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REACTIONS OF RAGWEED-SENSITIVE INDIVIDUALS TO SKIN TESTS WITH NUCLEIC ACIDS AND RELATED COMPOUNDS

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IN 1939, Winkenwerder, Buell, and Howard[1] published a preliminary report on the results of skin tests with nucleic acids and their derivatives on patients with ragweed hay fever. They found that persons reacting to ragweed pollen also gave positive skin reactions to dilute solutions of nucleic acids, nucleotides, purines, pyrimidines, caffeine, allantoin, and histidine, and in some cases, to proline and urea. In ten patients, skin-sensitizing antibodies to some of these compounds were demonstrable, and in two patients, severe constitutional reactions resulted from intracutaneous tests with nucleotides. On the basis of these results, they felt that nucleic acids and their derivatives bore a fundamental relationship to pollen sensitization. Lackman, Mudd, Sevag, Smolens, and Wiener[2] described precipitation of antipneumococcus horse serum by nucleic acids from various sources and pointed out that this precipitation had certain characteristics of an immunologic reaction. They found that nucleic acids did not cause precipitation in serum of patients sensitive to ragweed; but in making this comparison, one must bear in mind that the ragweed pollen antigen itself does not, under ordinary conditions, cause precipitation in such sera.

Although the results of Winkenwerder and his co-workers[1] aroused considerable interest at the time, no further details have been published and there has been no confirmation by others. Therefore, it seemed worth while to report briefly a study of the same sort.

Tests were carried out with preparations* of nucleic acid from yeast and from thymus and with adenine sulfate, allantoin, guanine hydro-

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*Samples of yeast and thymus nucleic acid were obtained through the courtesy of Dr. Stuart Mudd. Yeast nucleic acid was also obtained from Eimer and Amend and Merck & Co., Inc. The other pure chemicals were obtained from Eastman Kodak Co.

TABLE I

RESULTS OF DIRECT SKIN TESTS WITH NUCLEIC ACIDS AND OTHER NITROGEN COMPOUNDS

CASE NUMBER	YEAST NUCLEIC ACID	THYMUS NUCLEIC ACID	ADENINE SULFATE	ALLANTOIN	GUANINE HYDROCHLORIDE	HISTIDINE MONOHYDROCHLORIDE	PROLINE	UREA	XANTHINE	RAGWEED (100 P.N.* UNITS PER C.C.)	OTHER SENSITIZATIONS
Ragweed Hay Fever Cases											
1	0		0	0	0	±	0	0	0	+++	
2	0	0	0	0	0	0	0	0	0	++	Orris, dust
3	+	0	0	0	0	±	±	0	0	+++	Timothy, cottonseed, rice, orange
4	0		0	0	0	0	0	0	0	++	Timothy, cat, dog, dust
5	+++	+	0	0	0	+	0	0	0	++	Timothy, alternaria, dust
6	0	0	0	0	0	0	0	0	0	++	Feathers, dust, molds
7	+	0	0	0	0	+	0	0	0	+++	Timothy, plantain, sorrel, cocklebur, sage
8	+	0	0	0	0	+	+	0	0	++++	Timothy, trees, dust
9	0		0	0	0	0	0	0		++	
10	0		0	0	0	0	0	0	0	+	Timothy
11	0	0	0	0	0	0	0	0	0	++	
12	+	±	0	0	0	0	0	0	0	++++	Mugwort, dust
13	0	0	0	0	0	+	0	0	0	+++	Timothy, trees, dust
14	0	0	0	0	0	0	0	0	0	++	Timothy, dust
15	0	0	0	0	0	0	0	0	0	+	Dust
16	+++	++	±	+	++	+	±	±		++++	Many pollens
17	+		0	±	±	0	0	0	0	+++	Timothy, feathers, dust
18	0		0	0	0	0	0	0		+++	Timothy, amaranth, cocklebur
19	0	0	0	0	0	0	0	0	0	+++	Timothy
20	0	0	0	0	0	0	0	0	0	+++	Timothy, dog, dust, foods
21	+	0	0	0	0	0	0	0	0	++	Timothy, plantain, sorrel
22	+		0	+	0	0	0	±	±	+++	Dust, iva, mugwort
23	0	0	0	0	0	0	0	0	0	++	
24	0		0			0				+++	
25	++	++	+	++	++	+	++	±	+	+++	Dust, iva, mugwort
26	0	0	0	0	0	0	0	0	0	++	Cat, dog, dust
27	±	±	0		±	±				+++	Cocklebur, alternaria
28	+	+	0	0	0	0	0	0		+++	Timothy, plantain, sorrel, trees, dust
29	±	0	0	0	0	0	±	0	0	+++	
30	0	0	0	0	0	0	0	0	0	++	Dust, feathers
Allergies—Not Ragweed Hay Fever											
31	+	±	0	++	0	+	0	0	+	++	Timothy
32	0		0	0	0	0	0	0	0	0	Nupercaine
33	±		0	0	0	0	0	0	0	+++	Foods, dust
34	±		0	0	0	+	0	0	0	0	Amytal
35	0		0	0	0	0	0	0	0	0	Dust
36	+		0	0	0	0	0	0	0	0	Timothy, dust
37	+		0	0	0	0		0	0		Timothy, dust

Sixteen normal control cases all gave negative reactions and so are not individually tabulated.

*Protein nitrogen.

chloride, histidine monohydrochloride, proline, urea, and xanthine. In the case of the soluble compounds, 1:1000 solutions were prepared in 0.8 per cent sodium chloride solution. The compounds not soluble in water were dissolved by the addition of a small amount of sodium hydroxide, the final 1:1000 dilutions being near enough the neutral point so that they produced no irritation when tested on normal skin. The solutions were passed through Seitz filters, kept under sterile conditions, and tested by the intracutaneous injection of 0.01 to 0.02 c.c.

DIRECT SKIN TESTS

Intracutaneous tests were done on thirty patients with ragweed hay fever, seven patients with other allergic diseases (two of whom gave positive skin reactions to ragweed pollen, although not clinically sensitive), and on sixteen nonallergic normal persons. The reactions were read after ten to fifteen minutes and graded as follows: reactions with erythema and wheals 6 mm. or less in diameter, +; reactions with wheals 6 to 12 mm., without pseudopods, ++; those with wheals more than 10 mm., with pseudopods, +++; slight or questionable reactions, ±. Positive tests were checked and controlled with care to prove that they were not due to contaminated syringes or to dermatographia.

Of the thirty ragweed cases, eleven showed reactions to yeast nucleic acid, but only two were graded +++ and one as ++. Of twenty-two tested with thymus nucleic acid, two gave ++ and two gave + reactions. Among the seven patients with other miscellaneous allergic conditions, one gave a + reaction to yeast nucleic acid and three gave questionable (±) reactions. None of the sixteen normal control subjects gave any reaction to nucleic acids or to any of the other compounds tested. The cases are summarized in Table I.

Two of the patients (Cases 16 and 25) who reacted to nucleic acid also gave slight or questionable reactions to all of the other compounds tested. Both of these patients also gave skin reactions to a large number of pollens, many of which did not cause clinical symptoms, and to some of the inhalants. Aside from these two cases, there were only a few slight or questionable reactions to the purines, pyrimidines, amino acids, and other compounds tested.

INDIRECT SKIN TESTS (PASSIVE TRANSFER)

Sera were obtained from nine patients who had reacted on direct skin tests with one or more of the compounds, and passive transfer experiments were carried out according to the technique previously described at this clinic.[3] Three other sera (Cases 54, 55, and 56) from ragweed-sensitive patients who had not been tested intracutaneously with these compounds were also used for passive transfer. Some of the reactions obtained by direct tests were not demonstrable by the method

Sixteen normal control cases all gave negative reactions and so are not individually tabulated.
*Protein nitrogen.

of passive transfer, but five sera gave definite reactions to yeast nucleic acid and two to thymus nucleic acid (Table II). Although these reactions were perfectly definite and in many cases were demonstrated on two or more test subjects, none were large reactions and did not approach in size the reactions of the same sera to low ragweed pollen extract containing 1,000 protein nitrogen units or approximately 0.06 mg. of protein per cubic centimeter.

TABLE II

RESULTS OF PASSIVE TRANSFER TESTS WITH NUCLEIC ACIDS AND OTHER NITROGEN COMPOUNDS

CASE NUMBER	YEAST NUCLEIC ACID	THYMUS NUCLEIC ACID	ADENINE SULFATE	ALLANTOIN	GUANINE HYDROCHLORIDE	HISTIDINE MONOHYDROCHLORIDE	PROLINE	UREA	XANTHINE	RAGWEED (1,000 P.N. UNITS PER C.C.)	SENSITIZATION
3	+	+	0	0	0	0	0	0	0	+++	Ragweed
5	++	0	0	0	0	0	0	0	0	+++	Ragweed
7	0	0	0	0	0	0	0	0	±	+++	Ragweed
8	+	0	0	0	0	±	0	0	0	+++	Ragweed
13	±	0	0	0	0	0	0	0	0	+++	Ragweed
16	++	±	0	0	+	+	±	±		++++	Ragweed
25	+	+	±	0	+	++	+	±	+	++++	Ragweed
31	±	0	0	±	0	0	0	0	0	0	Timothy
37	0	0	0	0	0	0	0	0	0	0	Timothy
54	0	0	±	±	0	0	±	±		+++	Ragweed
55	0	0	0	0	0	0	±	±		+++	Ragweed
56	0	0	0	0	0	0	0	0		+++	Ragweed

Sera from the two patients who had shown many reactions on direct test also reacted to most of the same substances by the method of passive transfer, showing that the peculiarity of these two cases lay in the sensitizing antibody and not merely in the reactivity of the skin capillaries. Except for these two cases, there were no definite reactions to the simpler nitrogen compounds. Control tests on all the test subjects used for passive transfer were negative.

CROSS NEUTRALIZATION EXPERIMENTS

These were carried out with two sera which gave definite passive transfer reactions to nucleic acid. Portions of serum from Case 5 were mixed with equal volumes of yeast nucleic acid, 1:1000, low ragweed pollen extract, 1,000 protein nitrogen units per cubic centimeter, and 0.8 per cent sodium chloride. Two sites of each mixture were made in the back of a normal test subject and tested two days later with yeast nucleic acid (1:1000) and low ragweed pollen extract (1,000 protein nitrogen units per cubic centimeter), respectively. As shown in Table III, the addition of nucleic acid neutralized the reaction of the serum to this antigen but not to ragweed. On the other hand, the addition of ragweed pollen extract neutralized the reaction to nucleic acid

TABLE III

CROSS NEUTRALIZATION OF SERUM FROM CASE 5 WITH YEAST NUCLEIC ACID AND LOW RAGWEED POLLEN

MIXTURE NO.	SERUM OF CASE 5 AND	RESULTS OF TEST AFTER FORTY-EIGHT HOURS WITH	
		YEAST NUCLEIC ACID (1:1,000)	LOW RAGWEED POLLEN EXTRACT (1,000 P.N. UNITS PER C.C.)
1	Yeast nucleic acid (1:1,000)	0	++
2	Low ragweed pollen extract (1,000 P.N. units per c.c.)	0	±
3	Saline	+	++

TABLE IV

CROSS NEUTRALIZATION OF SERUM FROM CASE 16 WITH NUCLEIC ACID, HISTIDINE, AND GUANINE

MIXTURE NO.	SERUM CASE 16 AND	RESULTS OF TEST AFTER FORTY-EIGHT HOURS WITH		
		YEAST NUCLEIC ACID (1:1,000)	HISTIDINE (1:1,000)	GUANINE (1:1,000)
1	Yeast nucleic acid (1:1,000)	++	+	+
2	Saline	+++	+++	++

but did not (in this concentration) completely neutralize the reaction to ragweed pollen itself. In the case of serum from Case 16, on the other hand, addition of an equal volume of yeast nucleic acid solution (1:1000) did not neutralize the reaction to this substance or to histidine or guanine (Table IV).

DISCUSSION

The present results confirmed the fact that certain ragweed-sensitive patients react on skin test with nucleic acids, some of their derivatives, and other relatively simple nitrogen compounds, but the frequency and activity of these reactions were much less than would be expected from the report of Winkenwerder and co-workers.[1] Only about one-third of the ragweed cases reacted to nucleic acid, and still fewer to the other compounds tested, and in no case was the degree of reaction comparable to that produced by ragweed pollen. The amounts of the various compounds used for testing were as large or larger than those mentioned by Winkenwerder and much larger than the amounts of ragweed pollen protein which produced more marked reactions in the same patients.

The results did not suggest that nucleic acids or any of the other compounds studied were the chief determinants of specificity of ragweed pollen. The cross neutralization experiments, while not conclusive, did not suggest such a relationship. Other evidence leading to the same conclusion has been supplied by chemical analysis of highly active purified proteins separated from ragweed pollen by Stull and co-workers.[5] The absence of phosphorous indicated that these preparations did not

contain nucleic acid. The same studies also demonstrated that low ragweed pollen contains at least two distinct and specific antigenic proteins which must be considered in discussing the role of any chemical group as the determinant of the specificity of this pollen.

All but one of the ragweed-sensitive patients who reacted to nucleic acid also reacted to other pollens or various other antigens. This was particularly true of Cases 16 and 25. Some evidence[4, 6] has been offered that in such cases the many reactions are due to the presence of skin-sensitizing antibodies of rather broad reactivity rather than a multitude of strictly specific antibodies. Cross reactions of this type are perhaps due to some chemical relationship between the various antigens, but the significance of the relationship between ragweed pollen antigen and nucleic acid is minimized by consideration of the number of other antigens which gave reactions in the same patients, many of which were not clinically important. In fact, if reactions of the size produced by nucleic acid were produced in these same patients by one of the familiar antigens (pollens, inhalants, or foods), they would be considered of doubtful significance unless corroborated by the clinical history.

SUMMARY

1. Eleven of thirty ragweed-sensitive patients reacted to test with nucleic acid from yeast, but only three of these reactions were ++ or +++, and none were comparable to the reactions of the same patients to ragweed pollen extract.

2. Four of twelve patients reacted to thymus nucleic acid, and a few patients reacted slightly to guanine hydrochloride, adenine sulfate, histadine monohydrochloride, allantoin, proline, and xanthine.

3. In several cases, the reactions to nucleic acid were also demonstrated by the method of passive transfer.

4. The significance of these reactions was not apparent.

5. None of sixteen normal controls reacted to any of these compounds on direct skin test.

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THE CHEMISTRY OF ALLERGENS

VII. The Nature of the Unidentified Allergens of Cottonseed*

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INTRODUCTION

THE object of this investigation was the isolation and chemical characterization of the unidentified allergens (2CS†) of cottonseed. This progress report describes a method developed for guiding fractionation procedures and applications of this method in determining some properties of 2CS. Also described are two cottonseed-sensitive patients, encountered in this study, who were sensitive to 2CS alone but who were not sensitive to CS-1.

Coca and Grove,[3] in 1925, demonstrated that cottonseed contained more than one allergenic constituent. The isolation of the principal allergenic component of the cottonseed (CS-1[2] or CS-1A[4]) has been described in previous publications from this laboratory. It has also been demonstrated that the serum of some cottonseed-sensitive patients contained reagins for CS-1A alone, while the serum from other patients contained reagins for both CS-1A and 2CS.[5] Availability of fraction CS-1A, which was immunologically distinct from other allergens in the seed, provided a method for guiding further fractionation procedures.

METHOD OF CLINICAL ASSAY

The method employed for assaying the 2CS content of fractions from cottonseed was based on observations and procedures recorded by Coca and co-workers,[3, 6] Walzer and Bowman,[7] Cooke and Stull and their associates,[8, 9] and Lippard and Schmidt.[10] The principle involved was that under standardized conditions of testing, reagins can be specifically neutralized (in vitro) by one allergen leaving reagins for other allergens free to react independently in sites passively sensitized with serum-allergen mixture. (Sherman and Stull[11] reported that with certain

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*For Part VI of this series see reference No. 1.

†In previous papers a system of symbols was used to designate allergenic fractions. The generic symbol CS was adopted to describe fractions of cottonseed which possessed specificity characteristic of the first immunologically distinct fraction (CS-1[2]) isolated from the seed. In this paper the generic symbol 2CS is used to designate fractions possessing allergenic specificity distinct from that of CS-1. It is recognized that there may be several allergenic components as yet unidentified in cottonseed. It will be convenient to refer to them as 2CS, 3CS, etc., according to the chronologic order of their isolation or until they have been well enough defined to be given more descriptive names.

serums unrelated allergens sometimes effected cross-neutralization of reagins.)

Serums containing reagins for both CS-1A and 2CS were obtained from cottonseed-sensitive patients, L. C.[2, 5] and L. J.[5] Reagins for CS-1A were neutralized in vitro and the resulting mixture was used to prepare passively sensitized sites on the skin of subjects who exhibited no sensitiveness to cottonseed. Serial dilutions of cottonseed fractions, on a total nitrogen basis, were then injected into these sites after a suitable incubation period. The size of the wheal thus produced provided a means of assaying the 2CS content of the fractions.

MATERIAL USED

Whole Cottonseed.—A choice quality of dehulled cottonseed (*Gossypium hirsutum*) was ground and thoroughly extracted with benzene at room temperature. The solvent was removed from the seed by drying in a steam cabinet at temperatures not exceeding 50° C. The defatted seed was stored at –7° C. prior to use.

CS-1A.—The procedure used in the isolation of CS-1A from cottonseed has been described elsewhere.[4] CS-1A, which represented approximately 1 per cent of the defatted cottonseed, contained 11.8 per cent nitrogen and 39.9 per cent polysaccharidic carbohydrate. To neutralize reagins for CS-1A, however, a fraction CS-51R was used. CS-51R was obtained in an electrophoretic fractionation of CS-1A, which has been previously described.[12] CS-51R contained 19.8 per cent nitrogen and 0.9 per cent carbohydrate.

FRACTIONATION OF COTTONSEED

Description of all of the experiments conducted in determining the properties of 2CS cannot be presented here. The following experiment, however, is described to illustrate the clinical method of assay which was developed to guide fractionation procedures. Results, shown in Table I, illustrate a comparison of two globulin fractions to determine if heating at 85° C. for one hour destroyed 2CS activity. Details of the preparation and purification of globulin fractions are given later. The globulin fractions used in this test were obtained by the same general method which will be described later but were not as extensively purified. Cottonseed, previously leached with water, was extracted with 0.5 N. sodium chloride. The globulin in one-half of this salt extract was precipitated by pouring the extract into five volumes of water and, after being separated from the liquid, was redissolved in 0.5 N. salt solution. These steps were repeated four times. The final 0.5 N. salt solution of globulin contained 1.65 mg. per milliliter of nitrogen. The other half of the salt extract was heated on a water bath at 83-85° C. for one hour. The co-

agulum was centrifuged off and the globulin in the solution was precipitated and dissolved five times similarly to that in the unheated portion of extract. The final 0.5 N. salt solution of heated globulin contained 1.33 mg. per milliliter of nitrogen.

Aliquots of both preparations were sterilized with merthiolate 1:2000. For clinical tests, solutions were diluted to the desired concentrations in sterile physiologic salt solution containing merthiolate 1:10.[4] Solutions of equal nitrogen content were injected into passively sensitized sites in which reagins for CS-1A had been neutralized (in vitro). The results in Table I indicate that heating at 85° C. did not destroy the 2CS activity of the globulin. Quantitative determination of loss in 2CS activity would, however, require further tests with lower concentrations of the fractions.

TABLE I

COMPARISON OF 2CS ACTIVITY OF HEATED AND UNHEATED GLOBULIN FRACTIONS OF COTTONSEED

TEST SOLUTION	TOTAL NITROGEN CONTENT OF TEST SOLUTION (mγ*/0.025 ML.)	SENSITIZED† SITE NO.	RECIPIENTS‡ WHEAL DIMENSIONS IN MM.	
			M. E.	F. R.
Unheated globulin	100	1R	11 by 10	15 by 20
Unheated globulin	10	2R	9 by 8	12 by 12
CS-51R	100	3R	0	0
Heated globulin	100	4L	10 by 10	12 by 12
Heated globulin	10	5L	10 by 9	10 by 12
Controls				
CS-51R§	100	6L	22 by 20	21 by 25
CS-51R‖	100		0	0
Unheated globulin‖	100		0	0
Merthiolate‖ 1:10[4]			0	0

*The symbol mγ (millimicrogram) represents 0.000001 milligram. This symbol has been approved by the Committee on Nomenclature Spelling and Pronunciation of the American Chemical Society.[13]

†The sensitizing solution was prepared by mixing equal volumes of serum from cottonseed-sensitive patient L. J. with physiologic salt solution containing 500 mγ of CS-51R/ml. The mixture was allowed to stand at least over night at 5° C. before use. Sensitized sites prepared with this mixture gave no reaction to test with CS-51R as shown in site 3R.

‡Recipients were sensitized with 0.05 ml. of the mixture in three sites on the upper right arm and two sites on the upper left arm. Site 6L contained the serum salt mixture. After at least forty-eight hours the sites were injected with 0.025 ml. of the test solution as indicated. Reactions were read in fifteen to thirty minutes after the injection.

§Positive control. CS-51R was injected into site 6L which was sensitized with serum salt mixture.

‖Negative control tests on the unsensitized skin of the recipient.

Testing by this general procedure, it was demonstrated that 2CS, in contrast with CS-1A, was not dialyzable through cellophane membranes.* 2CS was also precipitable by basic lead acetate, whereas CS-1A was not precipitable. Heating globulin preparations at 100° appeared to destroy or remove at least 90 per cent of their 2CS activity.

Preparation of Cottonseed Globulin.—Accumulated evidence indicated that 2CS activity was owing to or closely associated with the globulin

*Cellophane tubing obtained from Fisher Scientific Co., and having a wall thickness of 0.00072 inches was used for dialysis experiments.

fraction of cottonseed. An attempt was therefore made to prepare a globulin fraction immunologically distinct from CS-1A.

Jones and Csonka[14] prepared globulin fractions from cottonseed by ammonium sulfate precipitation of sodium chloride extracts of the seeds. Their procedure was unsuited to the separation of CS-1A from the globulin, because CS-1A was soluble in the extracting solution and also was precipitated by the concentrations of ammonium sulfate required to precipitate the globulins. A method suggested by work of Olcott and Fontaine,[15] however, afforded excellent theoretical advantage for separating CS-1A from globulin. The principle of this procedure was first to extract exhaustively cottonseed with water in which CS-1A was soluble. The leached seed was then extracted with 0.5 N. sodium chloride to dissolve the globulin. Further purification of the globulin was accomplished by repeated reprecipitations effected by pouring the salt solution into five volumes of water.

Following is a description of the preparation and purification of two globulin fractions based on this procedure.

Unheated Globulin.—Fifty grams of defatted cottonseed were stirred with 400 ml. of distilled water and after standing for eighteen hours at room temperature, the suspension was centrifuged. Toluene was used as a preservative during the extractions. The supernatant solution was filtered through a folded paper and the total nitrogen content of the filtrate was determined. The seed was re-extracted nine times with water. Details of the extractions, which required a total of 251 hours, are collected in Table II. The nitrogen in the combined water extracts represented 29.3 per cent of the total. The tenth extract contained only 0.046 mg. per milliliter of nitrogen, indicating almost complete removal of water-soluble nitrogenous constituents.

The leached seed was then twice extracted with 0.5 N. sodium chloride solution which removed 27.6 per cent of the total nitrogen (Table II). The sodium chloride extracts were combined and poured into five volumes of distilled water. After standing nineteen hours the supernatant solution was decanted and the white globulin precipitate was recovered by centrifuging. This solid was redissolved in 400 ml. of 0.5 N. salt solution and reprecipitated as before. This process was carried out ten times. The nitrogen content of the supernatant solutions from these precipitations was 0.031, 0.008, 0.005, 0.005, 0.005, 0.007, 0.004, 0.002, 0.002, and 0.000 mg. per milliliter, respectively. The globulin fraction from the final precipitation was dissolved in 400 ml. of salt solution. This solution contained 1.182 mg. per milliliter of nitrogen which represented 43.5 per cent of the nitrogen present in the original salt extracts. The supernatant solutions from which the globulin was precipitated contained 18.7 per cent of the nitrogen present in the original salt

extracts, and the remaining 37.8 per cent was lost by denaturation and in handling. This preparation was designated globulin 2CS-1.

Notwithstanding the extensive purification process, this globulin still retained some CS-1A. When 1 ml. of the solution was injected into each of two guinea pigs sensitized to CS-1A, fatal anaphylaxis resulted in both cases. One milliliter of the globulin 2CS-1 solution injected into a normal guinea pig produced no effect.

TABLE II

EXTRACTION OF 50 GM. OF COTTONSEED* BY WATER FOLLOWED BY 0.5 N. SODIUM CHLORIDE EXTRACTION

EXTRACT NUMBER	SOLVENT	VOL. ADDED (ML.)	VOL. REMOVED (ML.)	EXTRACTED† HOURS	TOTAL NITROGEN CONTENT OF SOLUTION (MG./ML.)	TOTAL NITROGEN REMOVED (%)
1	Water	400	320	18	2.24	18.2
2	Water	320	315	1	0.53	22.4
3	Water	315	295	24.2	0.28	24.5
4	Water	320	300	20	0.13	25.5
5	Water	320	330	3	0.076	26.1
6	Water	320	315	21.7	0.083	26.8
7	Water	320	295	73.9	0.148	27.9
8	Water	320	310	19.5	0.078	28.5
9	Water	320	305	47	0.058	28.9
10	Water	320	300	22.0	0.046	29.3
11	0.5 N. NaCl‡	320	300	3	2.42	47.8
12	0.5 N. NaCl	300	330	1	1.09	56.9

*Contained 7.8 per cent total nitrogen.

†This period represents time of standing or of gentle agitation.

‡Sufficient sodium chloride was added to make the residual water present in the leached seed 0.5 N.

Heated Globulin.—Jones and Csonka[14] have shown that a "phospho protein" was precipitated when salt extracts of cottonseed were heated to 85° C. According to these authors, higher temperatures were required to coagulate the globulin fractions. Further purification of globulin 2CS-1 was therefore attempted by heating 200 ml. of the previously described salt solution on a water bath at 83 to 85° C. for one hour. After cooling, the coagulum was removed by centrifuging. The solution (191 ml.) contained 0.655 mg. per milliliter of nitrogen or 53 per cent of the nitrogen originally present. The heated solution was precipitated by being poured into five volumes of water. The white solid was recovered and dissolved in 100 ml. of 0.5 N. salt solution and reprecipitated four times as before. The process was then repeated ten more times, using 40 ml. of 0.5 N. salt solution to dissolve the globulin. The total nitrogen content of the supernatant solutions from these precipitations ranged from 0.006 mg. per milliliter for the first to 0.002 mg. per milliliter for the fifteenth precipitation. The final precipitate was dissolved in 57 ml. of 0.5 N. salt solution. This solution contained 0.498 mg. per milliliter of nitrogen and was designated globulin 2CS-2.

Globulin 2CS-2 was also not completely free from CS-1A antigen. When 0.6 ml. of the solution was injected into each of three guinea pigs sensitized to CS-51R, slight delayed anaphylaxis resulted in two cases. These two animals suffered fatal anaphylaxis when subsequently tested with CS-51R. The third pig was not affected by the globulin 2CS-2 injection, but severe anaphylaxis resulted from injection of CS-51R.

Because of an indefinite interruption of this phase of this investigation soon after the preparation of these globulin fractions, their 2CS activity was not studied by the procedure illustrated in Table I. These preparations were, however, used for intracutaneous testing of the two 2CS-sensitive patients described below.

2CS-Sensitive Patients.—It was recognized early in the investigation which resulted in the isolation of CS-1A that cottonseed contained more than one skin-reactive factor. Five patients originally classified as cottonseed sensitive were soon excluded as being unsuitable for guidance in chemical fractionation procedures because of unexplained irregularities in their skin response to the fractions. Later these patients (Type I)[2] were found to react negatively to CS-1A, and their serum did not contain reagins for cottonseed. Selection of a group of patients on the basis of uniformity of their skin reaction for guidance of fractionation procedures led to the isolation of CS-1A. Patients exhibiting cutaneous sensitivity to CS-1A (Type II)[2] were uniformly clinically sensitive to cottonseed, and, with one exception, their blood serum contained reagins for CS-1A. In the present study, therefore, patients were routinely tested with CS-1A or CS-51R to ascertain their lack of sensitiveness to cottonseed and thus their suitability to serve as recipients for passive transfer tests. Routine selection of recipients by this method led to the incidental discovery of a patient who was cutaneously sensitive to 2CS and whose blood serum contained reagins for 2CS but not for CS-1A. Patient E. M., who reacted negatively to CS-51R, was used as a recipient in a cross neutralization experiment designed to determine if an intermediate globulin preparation was immunologically free from CS-1A. This patient was sensitized in two sites (1R and 2R) on the upper right arm with L. B. serum neutralized with CS-51R. Two sites (3L and 4L) on the left arm were sensitized with L. B. serum neutralized with the globulin preparation. An additional site (5L) on the left arm contained the usual serum-salt mixture to serve as control. Results of this test are shown in Table III. Site 1R gave a positive reaction when tested with globulin. Although this reaction was smaller than the control, it indicated sensitiveness of the recipient as well as passive sensitiveness of the site conferred by the L. B. serum. Site 2R when tested with CS-51R gave a negative reaction, indicating that reagins for CS-51R in L. B. serum had been neutralized. Site 3L was positive owing to sensitiveness of the recipient. Site 4L was

negative because the globulin preparation contained sufficient contaminating CS-51R to neutralize the corresponding reagins. Control site 5L gave a positive reaction owing to the passive sensitivity of the skin. The control site into which the globulin was injected into the normal skin gave a large wheal with immediate itching and redness owing to the natural sensitiveness of the patient to this fraction. *Thus the specificity of two allergenic components from cottonseed was demonstrated on a single patient by a simultaneous positive passive transfer test with one allergen and a positive direct test with a different allergen.*

TABLE III

SIMULTANEOUS PASSIVE TRANSFER TEST WITH CS-51R AND DIRECT INTRACUTANEOUS TEST WITH A COTTONSEED GLOBULIN PREPARATION ON PATIENT E. M.

TEST SOLUTION	TOTAL NITROGEN CONTENT OF TEST SOLUTION (mγ*/0.025 ML.)	SENSITIZED SITE NO.	PATIENT E. M.‖ (WHEAL DIMENSIONS IN MM.)
Globulin	100	1R†	12 by 10
CS-51R	100	2R†	0
Globulin	100	3L‡	9 by 7
CS-51R	100	4L‡	0
Controls			
CS-51R	100	5L§	15 by 14
Globulin	100	Normal skin	18 by 14

*See footnote (*) to Table I.

†Sensitizing solution for sites 1R and 2R was prepared by mixing equal volumes of L. B. serum with physiologic salt solution containing 500 mγ of CS-51R/ml.

‡Sensitizing solution for sites 3L and 4L was prepared by mixing equal volumes of L. B. serum with physiologic salt solution containing 500 mγ of globulin per milliliter.

§Sensitizing solution for site 5L was prepared by mixing equal volumes of L. B. serum and physiologic salt solution.

‖E. M. was sensitized with 0.05 ml. of each mixture. After ninety-six hours the sites were injected with 0.025 ml. of the test solutions as indicated. Reactions were read fifteen to thirty minutes after injection.

TABLE IV

PASSIVE TRANSFER TESTS WITH SERUM FROM 2CS-SENSITIVE PATIENT E. M.

TEST SOLUTION	TOTAL NITROGEN CONTENT OF TEST SOLUTION (mγ†/0.025 ML.)	RECIPIENTS‡ WHEAL DIMENSIONS IN MM.		
		L. P.	M. P.	J. R.
CS-51R	100	0	0	0
Globulin	100	9 by 9	11 by 8	10 by 10
UCS-S*	100			9 by 10
Controls				
Globulin	100	0	7 by 5§	0
UCS-S	100			0

*UCS-S represents an unfractionated 5 per cent sodium chloride extract of cottonseed.

†See footnote (*) to Table I.

‡Recipients were uniformly sensitized on the right upper arm with 0.05 ml. of E. M. serum. After at least forty-eight hours the indicated quantity of cottonseed preparation was injected in 0.025 ml. of sterile solution. Reactions were read fifteen to thirty minutes after injection of the test solution.

§No heat in this control test.

The blood serum of E. M. contained reagins for 2CS but not for CS-51R, as shown in Table IV. The passive transfer reactions obtained were typically positive but were not nearly as large as those produced

by CS-51R with serums from Type II cottonseed-sensitive patients. However, no reagins to either allergen were demonstrable in the serum of another patient, W. E., who reacted positively to cutaneous tests with unfractionated cottonseed but gave negative reaction to tests with solid CS-1A.

TABLE V

INTRACUTANEOUS TESTS ON 2CS-SENSITIVE PATIENTS SHOWING SPECIFICITY OF THE SKIN RESPONSE TO CS-51R AND 2CS FRACTIONS OF COTTONSEED

TEST SOLUTION*	TOTAL NITROGEN CONTENT OF TEST SOLUTION (mγ†/0.025 ML.)	SITE‡ NO.	PATIENT§ WHEAL DIMENSIONS IN MM.	
			E. M.	W. E.
CS-51R	10,000	1	0	11 by 1[illegible]
Globulin 2CS-1	100	2	14 by 11	15 by 11
Globulin 2CS-2	100	3	0	5 by 6
UCS-0.5 NS‖	100	4	16 by 15	20 by 15
Merthiolate 1:10⁴	-	5	0	8 by 6

*Stock test solutions were made up in 0.5 N. sodium chloride solution and sterilized with merthiolate 1:2000. To obtain the desired concentrations for testing the stock solutions were diluted into sterile 0.5 N. sodium chloride solution. The final concentration of merthiolate was 1:10,000 in all test solutions.

†See footnote (*) to Table I.

‡0.025 ml. of each solution was injected intracutaneously into each site.

§E. M. (Fig. 1); W. E. (Fig. 2). Reactions were read and photographs were made fifteen to thirty minutes after injection of the test solution.

‖Unfractionated 0.5 N. sodium chloride extract of cottonseed.

Conclusive evidence of the specificity of the skin response of two patients, E. M., and W. E., to CS-51R and 2CS was shown by direct testing with a series of these fractions. The same solvent was used for all of the test solutions which were injected in equal volume. Results of these tests are shown in Table V and in the three illustrations. Fig. 1 shows results of the test on patient E. M. Injection of 10,000 mγ of CS-51R nitrogen at site 1 gave no reaction as compared with the control test, site 5. However, only 100 mγ of globulin 2CS-1 nitrogen injected at site 2 produced a 14 by 11 mm. wheal with heat and a 30 by 30 mm. zone of redness. Site 3, which received 100 mγ of heated globulin 2CS-2 nitrogen, gave no reaction as compared to the control. Site 4 into which was injected 100 mγ of nitrogen of an unfractionated 0.5 N. salt extract of cottonseed gave a 16 by 15 mm. wheal with heat and a 50 by 50 mm. zone of redness.

A similar series of tests was conduced on patient W. E. with qualitatively similar results (Fig. 2). However, the reaction of W. E. to 100 mγ of nitrogen from the unfractionated 0.5 N. salt extract of cottonseed, site 4, was significantly larger than the reaction produced by an equal quantity of globulin 2CS-1 nitrogen in site 2. This indicated that W. E. was sensitive to another unidentified component of cottonseed or possibly that the globulin was not inherently active in this case but owed its activity to a contaminant.

The specificity of the skin response of patient W. E. was further demonstrated by tests with solid CS-1A and whole cottonseed (Fig. 3). The

negative reaction to solid CS-1A is shown at site 1, while the wheal produced by whole cottonseed is shown at site 2. Patients sensitive to CS-1A (Type II) give large wheals in a few minutes when tested with $1:10^5$ to $1:10^6$ dilutions of CS-1A.

Fig. 1.—Intracutaneous tests on 2CS-sensitive patient E. M. Each site was injected with 0.025 ml. of a 0.5 N. sodium chloride solution containing merthiolate 1:10,000 and cottonseed allergenic fraction as indicated: *1*, 10,000 mγ CS-51R nitrogen; *2*, 100 mγ globulin 2CS-1 nitrogen; *3*, 100 mγ heated globulin 2CS-2 nitrogen; *4*, 100 mγ unfractionated cottonseed nitrogen; *5*, control.

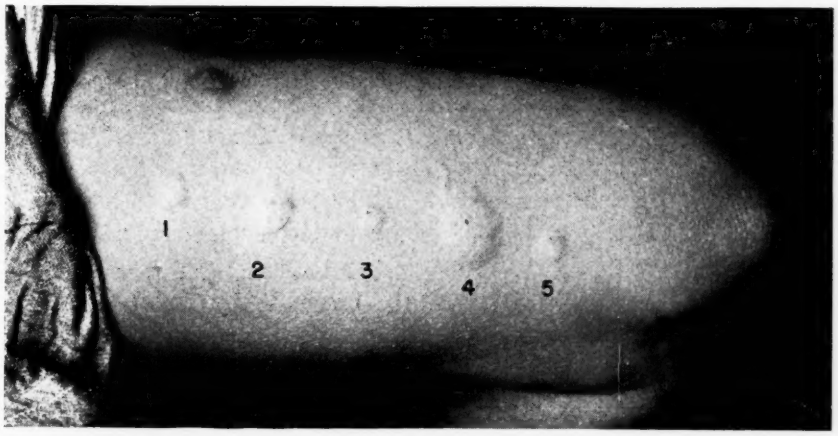

Fig. 2.—Intracutaneous tests on 2CS-sensitive patient W. E. Each site was injected with 0.025 ml. of a 0.5 N. sodium chloride solution containing merthiolate 1:10,000 and cottonseed allergenic fraction as indicated: *1*, 10,000 mγ CS-51R nitrogen; *2*, 100 mγ globulin 2CS-1 nitrogen; *3*, 100 mγ heated globulin 2CS-2 nitrogen; *4*, 100 mγ unfractionated cottonseed nitrogen; *5*, control.

Brief histories of these Type I patients are as follows:

CASE 1.—E. M., colored female, aged 31 years. Family history negative for allergy; has had perennial asthma, which is more severe in March, for one year. Cutaneous tests positive for house dust, paper mulberry pollen, feathers, and cottonseed.

CASE 2.—W. E., colored male, aged 27 years. Family history negative for allergy; has had perennial asthma, which is worse in summer, for four years. Cutaneous tests positive for grass pollens, horse dander, clam, house dust, and cottonseed.

Fig. 3.—Cutaneous test on 2CS-sensitive patient W. E.: *1*, Solid CS-1A; *2*, whole cottonseed.

DISCUSSION

Separation of one allergen of cottonseed, immunologically distinct from other allergens, has made available a method for biologic assay of the unidentified active components. After neutralization of reagins for CS-1A in serums of certain cottonseed-sensitive patients, the reagins for the unidentified constituents were available for reaction in passively sensitized sites. The residual reagins in serum from different patients may, however, vary with respect both to number and specificity. Moreover, the situation is further complicated by the possibility of nonspecific cross neutralization by certain of the unidentified components.[11]

The recognized limitation of the neutralization method for guiding fractionation could to some extent be overcome by direct testing on Type I patients who are sensitive to the unidentified allergens alone but who are not sensitive to CS-1A. Tests on patients of this type could be used for clinical guidance of fractionation procedures similar to those employed in the isolation of CS-1A. Two such cottonseed-sensitive patients were discovered in this investigation, but attempts to use them for guidance in chemical fractionation procedures have not yet been made. It is also recognized that the sensitivity of 2CS-sensitive patients may be owing to allergens of different identity than those which react with reagins in CS-1A neutralized serum. That different allergens are

responsible for these two reactions is indicated by the fact that heating a globulin preparation to 85° C. destroyed its wheal-producing capacity on 2CS-sensitive patients, E. M. and W. E. Heating, however, did not appear to affect the wheal-forming capacity of the globulin in sites passively sensitized with CS-1A neutralized serum (Table I). Available evidence does not exclude the possibility that 2CS activity of the globulin preparations was owing to the presence of a difficultly removable contaminant. The persistence with which CS-1A remained in the globulin preparations, as shown by anaphylactic tests, demonstrated the difficulty of eliminating traces of contaminants from them.

The indication that 2CS activity was owing to the globulin fraction of cottonseed was of particular interest because of the possible relationship of proteins containing relatively high proportions of arginine to allergenic activity. More than 20 per cent of the total nitrogen content of the cottonseed globulins is arginine nitrogen.[16, 17, 18] Mitchell and Hamilton[19] pointed out that proteins of nuts and certain oil-bearing seeds were uniformly rich in arginine (20 per cent of the total nitrogen), moderately rich in lysine, and relatively poor in histidine. For the most part, these arginine-rich proteins were globulins. The clinical observation that most cottonseed-sensitive patients are also sensitive to nuts and certain oil-bearing seeds has been frequently made and previously recorded by Bowman and Walzer.[20] Allergens from these sources are of the highest potency of any from vegetable sources. As high as 33 per cent of the total nitrogen content of fraction CS-51R was found to be arginine nitrogen.[21] It was therefore previously suggested that coexistent sensitiveness to cottonseed, nuts, and oil-bearing seeds was possibly due to proteins of high arginine content like CS-51R.

It seems possible also that globulins of nuts and other oil-bearing seeds may be allergenically analogous to the globulin of cottonseed.

SUMMARY

1. A method for guidance in chemical fractionation procedures and an attempted isolation of unidentified allergens in cottonseed are described.

2. Globulin fractions of cottonseed, which retained 2CS allergenic activity after prolonged purification, were prepared. These purified globulin fractions were not completely free from CS-1A, as shown by anaphylactic tests.

3. Conclusive evidence of the specificity of the skin response of cottonseed patients to CS-1A and 2CS was shown by direct testing with a series of fractions. Two Type I patients reacted positively when tested with whole cottonseed or unheated globulin preparations but negatively when tested with CS-1A or CS-51R.

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CUTANEOUS REACTIONS AS AN INDEX TO DESENSITIZATION TO HISTAMINE

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I. INTRODUCTION AND HISTORICAL RÉSUMÉ

SINCE the isolation and chemical identification of histamine (B-iminazolylethylamine) from ergot by Barger and Dale,[1] a great deal of investigation has been done with the drug from chemical, pharmacologic, physiologic, and clinical standpoints. In this paper I propose to deal primarily with one phase of the amine's activity; namely, the response of the skin to intradermal injections. It is our purpose to describe changes in the cutaneous response to histamine after repeated subcutaneous injections of gradually increased amounts.

The effect of histamine on the skin was first noted by Eppinger[2] in 1913. Sollman and Pilcher,[3] in 1917, experimenting mainly on themselves, produced local reactions to histamine which were identical with Eppinger's findings. Their methods were the scarification technique, or "bloodless vaccination," and the Mucuna technique. The latter consisted of rubbing gently on the skin, to which histamine had been applied, cowhage, or Mucuna, for one minute. This material consists of stiff hairs from leguminous seed pods. They found that the amine produced a wheal up to 1:500,000 dilution; that a 1:5,000,000 dilution had no effect. It must be remembered, when considering the dilutions I used, that Sollman and Pilcher did not use the intradermal injection technique. They described the resultant wheal, itching and reddening (flare), but drew no further conclusions.

The Triple Response.—The work of Lewis and his colleagues[4] is most illuminating and will be considered in some detail. They described the skin reaction as a "triple response"; namely, (1) vasodilatation of the small vessels at the site of injection; (2) a diffuse flare; and (3) an area of local edema or wheal which appeared over the small area exhibiting the vasodilatation. The vasodilatation and wheal are due to direct action on the capillary wall; the wheal is caused by an increased permeability of the capillary wall with resultant exudation of fluid into the tissue spaces. The flare is caused by a local nerve reflex and is obtained up to five to seven days after section of the peripheral nerve, before degeneration sets in. The mechanism of this reflex will be considered later.

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This reaction has been observed for many years to follow a wide variety of dermal stimulants. When the skin affected by the flare was examined under the microscope, Krogh[5] found that the capillaries and venules were widely dilated and that the current of blood flow was accelerated. Carrier[6] (1922) introduced histamine in 1:1,000 dilution into the skin by means of a glass capillary and noted at the nail base widening and hastening of the stream. The latter is due principally to arteriolar dilatation. For this reason the flare is sometimes called "arteriolar flare." Because of the nervous mechanism provoking it, it is often called "reflex erythema."

Müller,[7] in 1913, first drew attention to the difference in skin response to histamine in sensitive and insensitive skin. He stated the arteriolar flare was unobtainable in skin deprived of its nerve supply through injury to the nerve trunk and in areas corresponding to injuries of the spine and cord. He therefore concluded that the flare was brought about through a spinal cord reflex. His evidence, however, was incomplete, as was shown by the later work of Lewis, for he failed to show that involvement of the posterior root ganglia or sensory nerves leading to degeneration would be equivalent to section of a peripheral nerve. Breslauer[8] (1919) observed this flare after application of mustard oil to the skin and noted its occurrence only when the peripheral nerves were intact, and its absence when the skin was anesthetized with novocain. Lewis[10] demonstrated that the flare occurs even when the corresponding section of the spinal cord is destroyed if the posterior root ganglion is left intact. The reflex, then, is strictly a local one not depending on the spinal cord. Even if the nerve trunks to a part are severed, the reaction is still obtainable *until* degeneration sets in. The reflex, therefore, must involve a distinctly local nervous mechanism, and the conception of this mechanism is as follows: stimulation of the end-organs of the fibrils of a single nerve fiber results in a spread of the impulse to all the branches of that fiber and causes relaxation of the capillary and arteriolar walls with which it is connected. Local vasodilatation and wheal formation occur in areas long after degeneration of their peripheral nerves, and, therefore, do not depend upon the integrity of this nervous mechanism but rather on direct stimulation of the cells of the vessel walls by the irritating agent.

Because of the rise in skin temperature of the area exhibiting the flare, Lewis[10] concluded that blood flow is increased and that arterioles must be involved. This was substantiated by the fact that he could produce a flare of arterial color in an extremity in which the venous circulation was occluded and the skin was bluish; also by the fact that when the arterial circulation was occluded, no flare was produced, but when the circulation was opened, it appeared, following the subsidence of the general reactive hyperemia. This was taken to prove that the

capillaries and venules had no *active* part in production of the flare, for, if they did, it should have appeared *during* the arterial occlusion. Krogh[5] disagreed on this one point, believing the minute vessels do play their part. He stated that it is impossible passively to open the smaller vessels by arteriolar dilatation alone, for a flow of almost any magnitude brought about by arteriolar dilatation can be taken up by the capillaries and venules without appreciable dilatation.

As regards the exact path of the reflex, there are contradictory views. Török and Rajka[9] (1925) found that the injection of novocain under the true skin, though producing anesthesia, failed to inhibit the erythema. This would lead one to believe that the pathways were superficial, running in or close to the corium. Lewis, Grant, and Marvin[10] (1927), however, stated that the reflex does not pass through the true skin because they could not inhibit it by superficial incisions (3 to 4 mm. through the skin and 2 cm. in length). The flares in these cases passed beyond the barriers. Krogh[5] brings out the interesting opinion that perhaps the reflex had spread around or about these barriers, traveling by way of the intricate web of nerve fibers in the skin. He substantiated this point by a clever experiment in which he completely surrounded the site of injection by four superficial incisions and noted in general no passage of the flare beyond the incisions. He concluded that the nerve fibers for the most part traveled in the superficial layers of the skin. As to the exact nature of the pathways used, it is the common belief that the peripheral sensory fibers are responsible, and, of these, most likely pain-carrying ones.

There is a great similarity between the histamine response described above and that produced by mechanical irritation of the skin and by application of a wide variety of chemical stimulants. Lewis and Harmer[11] postulated that these latter reactions were due to liberation of a histamine-like substance, probably histamine itself, from injured tissue cells. In patients with urticaria factitia they could produce, by extensive stroking of the skin over large areas of the body, systemic reactions practically identical to those following the administration of histamine, namely, flushing of the face and increased skin temperatures. The blood pressure and pulse rate remained about the same. To corroborate this evidence, Harris[12] (1927) succeeded in showing that alcoholic extracts of the human skin (obtained at surgical operations) contained a substance which acted like histamine and which was not acetylcholine. He found that normal skin contained about 1:100,000 parts of this histamine-like substance and that it was present in the epidermis in quantities six times that contained in the dermis. Orr[13] stated that the histamine content of epidermis was 24 mg. per kilogram (equivalent to a 1:40,000 solution), and that it was not present in injured skin in greater amounts than in intact skin but disappeared from the injured

skin in a short time due to absorption by the blood. If the amount absorbed was adequate, a systemic response occurred.

The Histamine Skin Reaction in Pathologic Conditions.—The skin reaction to histamine has been used as an aid to diagnosis in a wide variety of pathologic states of the human organism. The value of some of the conclusions is questionable, and the actual aid the test affords is uncertain. Several workers have noted changes in the way of reduction in size of wheals or a delayed or absent flare in circulatory deficiencies. King[14] tested the skin with histamine in patients with dermatophytosis, alopecia areata, and scleroderma. McConnell[15] noted larger wheals during allergic manifestations (hay fever, asthma, etc.). Caldwell and Mayo[16] used the test in Raynaud's disease, scleroderma, and chronic infectious arthritis with inconclusive results. Loeser[17] put to clinical use the known fact that the flare depends on nerve supply peripheral to the posterior root ganglia and sensory nerve roots and used the test to detect true dermal anesthesia. Most of the workers have used the wheal as the diagnostic criterion rather than the flare, and King said the flare was not a good criterion as the size varied so greatly in each individual and in different individuals. However, he used the scarification technique with one drop of a 1:1000 solution of histamine acid phosphate, pricking into the skin seven times with a needle. Attempt to compare flare sizes with such a method seems inaccurate, for the method is qualitative and the conclusions quantitative. It seems that in most of the pathologic states mentioned there are more valuable and definite diagnostic criteria at hand to aid the physician than the histamine skin test.

Ernstene and Snyder[18] noted the effect of arteriosclerosis and benign and malignant hypertension on the area of histamine flares. They abandoned the puncture method and used the injection technique. Their experiments were well carried out and effectively presented. Their results were: (1) With 0.02 c.c. of 1:2000 solution of histamine dihydrochloride injected intradermally, the average flare produced in thirty normal individuals was 31 sq. cm. in area. The injections were not done in a constant temperature room. There was not much variation in size between any of the normal flares. (2) In patients with arteriosclerosis and normal blood pressure there was slight reduction in the size of flares, 18 to 22 sq. cm. (3) In benign essential hypertension the area was within normal limits, averaging 29 sq. cm. (4) In intermediate hypertension the average flare was 20 sq. cm. (5) In malignant hypertension, it was 16 sq. cm. They postulated that the reductions in area of flare were due to changes in the size of the lumen of the arterioles, either organic changes in the walls, or functional, spastic states. They believed that these observations might be put to use as diagnostic criteria in the case of malignant hypertension where other tests might be inconclusive.

Skin Immunity.—There are contradictory views as to whether repeated applications of histamine to the skin produce "immunity." Sollman and Pilcher[3] (1917) found that repeated injections whether to the same or to the opposite arm produced smaller wheals in the later applications. There was no mention of any change in the size of the flares. Ebbecke[19] (1923) stated that the reactions from histamine were not reduced, while those of the specific poisons (nettles, etc.) induced a more or less complete "immunity."

Summary.—When histamine is injected into the skin there is elicited a triple response; namely, (1) vasodilatation of the small vessels at the site of injection, (2) a diffuse flare, (3) an area of local edema or wheal. The vasodilatation and wheal are the results of direct action of histamine on the capillary wall. The flare is caused through stimulation of peripheral nerves (probably pain fibrils) and consequent arteriolar and minute vessel dilatation. These nerves probably travel through the superficial layers of the skin. The response depends upon the integrity of the nerves from posterior root ganglion to the periphery. The mechanism involved is strictly a local reflex. The histamine skin response has been used in a wide variety of pathologic states as a diagnostic aid, but its value is questionable.

II. THE PROBLEM OF HISTAMINE DESENSITIZATION

The problem of histamine desensitization is a relatively new one. It is not established definitely as yet whether or not such a state exists. There is good evidence to indicate that many of the allergic manifestations are due to liberation of histamine or histamine-like substances from the tissues involved. Giant urticaria has been treated successfully with histamine. It has been suggested that cold allergy represents a histamine-like reaction (Horton and Brown[21] and Levine[22]) and that it may be treated with success by repeated injections of histamine in increasing dosages. Bray[23] reported a case of a boy sensitive to cold who experienced systemic histamine-like reactions and marked swelling and redness of the hand immersed in water at 45 degrees. Successful immunity was produced to this type of physical allergy by repeated injections of histamine. Intermittent hydrops and periodic edema have been treated in similar fashion. Mueller[24] reported the case of a woman with periodic edema of the left hand occurring every fourteen days and lasting seven days who received repeated increasing amounts of histamine with resulting temporary relief for five weeks, at which time the edema recurred and a second course of injections was given; eleven months later she was still free of edema.

These instances show the favorable results obtained with histamine in some states of allergy. With this thought in mind, Roth and Rynearson[25] treated insulin hypersensitivity with repeated injections

of the amine and concluded that this procedure was the quickest and best cure of the insulin allergic state.

This evidence supports the theory that histamine desensitization can be accomplished. This is not direct evidence. It does show, however, that in pathologic conditions in which there is reason to suspect histamine as the cause, repeated injections of the drug in some way alter the physiology of the organism so that the pathologic condition no longer exists. For want of a more exact definitive term pertaining to this state, I use the word "desensitization." I do not intend this to imply an antigen-antibody reaction but merely a state of altered response of an organism to a specific substance, the basis of which is not yet understood. It is not within the scope of this paper to deal in detail with the exact mechanism involved.

III. PRELIMINARY INVESTIGATIONS AND METHODS

The patients used in the experiments were obtained from various sections of the Mayo Clinic. Most of them were suffering from headache which could be classified as a vasodilating or erythromelalgic type. This syndrome has been described by Horton, MacLean, and Craig.[26] The characteristic symptoms and physical and laboratory findings are as follows: (1) Hemicrania. The headache usually is one-sided, but may progress during its course to become generalized; (2) onset is usually later in life than the typical migraine which so frequently begins in childhood or adolescence; (3) precipitation of a typical headache may be effected by vasodilating agents such as alcohol, heat, and histamine; (4) relief by vasoconstricting agents such as epinephrine or cold; (5) increased skin temperature of the side of the head affected during an attack of pain; (6) signs of local vasodilatation over the area of pain, such as flushing, sweating, lacrymation, rhinorrhea; (7) relief of pain by manual pressure over the temporal or carotid artery (inconstant); (8) frequent occurrence while the patient is lying down (increased blood supply to the head) or exaggeration of an already existing headache by change to the supine position; (9) absence of definite migrainous periodicity, nausea and vomiting, scotomas.

It was proposed by Horton, MacLean, and Craig that this type of headache was due to histamine vasodilatation. In patients exhibiting this syndrome most of the characteristic symptoms (and sometimes all) can be produced by the injection subcutaneously of 0.1 to 0.7 mg. of histamine. The headache that results is indistinguishable from the patient's own spontaneous headache, and there is a rise in skin temperature over the side of the head corresponding to the location of the pain. Many writers have produced evidence that some headaches are due to local vasodilatation. Clark, Hough, and Wolff[27] produced headache by the intravenous injection of 0.1 mg. of histamine phosphate and simultaneously recorded the blood pressure, temporal pulsations,

and intracranial pulsations by an ingenious experimental set-up. Their conclusions, after careful evaluation of their own work and that of many other investigators in the field, were as follows: the headache produced by an injection of histamine was a result of dilatation of the cerebral vessels; the headache occurred at the moment when the blood pressure began to rise after its initial fall after the injection, and not during the fall. They believed, therefore, that the cerebral vessels, being in a state of relaxation when this rise in blood pressure occurred, could not adequately accept the new burden of increased magnitude of flow, and sensory end organs in their walls were stimulated, resulting in painful sensation. When the intracranial pressure was raised to certain limits, this effect was neutralized, causing diminution or disappearance of the headache. Pickering[28] drew similar conclusions. Balyeat and Rinkel[29] concluded that migraine as a whole is caused by local vasomotor dilatation, producing congestion of the brain corresponding to a hive on the skin. They classified the disease, therefore, as allergic in origin.

Other patients used in our experiments were receiving histamine treatment because of allergic manifestations that did not respond to routine methods, such as vasomotor rhinitis, urticaria, drug sensitivity, etc.

These patients received the usual course of histamine treatment given at the clinic which consists of small subcutaneous injections of histamine acid phosphate in gradually increasing dosages twice daily for a period of ten days to two weeks. The patients remained at the clinic during this time and were observed from day to day. The initial dose consisted of 0.25 c.c. of histamine acid phosphate in dilution of 0.19 mg. per cubic centimeter, or the equivalent of 0.025 mg. of histamine calculated as histamine base. This dosage was increased at each injection by 0.05 c.c. until 1 c.c. was reached, when it was maintained at that level. If systemic reactions occurred after injection (flushing, fall in blood pressure, severe headache), the amount of histamine was reduced and gradually increased. Most of the patients exhibited no reactions to these small dosages.

It was necessary to control the experiments carefully, realizing the physiologic basis for the skin response which has been described earlier. Since it has been definitely shown that the flare depends on arteriolar and minute vessel dilatation, it would seem that there should be a difference in the size of flares obtained in skin at different temperatures. To show this definitely, three normal subjects were tested in the following manner. The subject was placed supine in a constant temperature room at 86° F. and 40 per cent relative humidity. Thermocouples were applied to four areas on the arm and forearm, and temperature readings were taken until constant. Then the thermocouples

were removed and intradermal injections were performed, using histamine in dilutions of 1:100,000, 1:1,000,000, 1:4,000,000 and 1:8,000,000 in the exact areas to which the thermocouples had been applied. At maximal flaring, the flare zones were recorded on tissue paper and their areas calculated by the methods presently to be described. Then the subject was moved to a room at 78° F. and 40 per cent relative humidity and the exact procedure duplicated. This was done a third time in a room at 68° F. and 40 per cent relative humidity. For the second series of injections I used the other arm and then by the time the individual was ready to be placed in the cold room (68° F.) the reactions on the first arm had entirely disappeared, making it possible to use that one for the third series. One of the subjects was started in the warm room (78° F.), then moved to the hot room and then to the cold room. In the normal subject I could detect no difference in the skin reactions of either arm to the same amount of histamine. The results of this experiment are recorded in Table I, and the data concerning Subject 1 are graphically depicted in Fig. 1. There was a marked difference in size of flares taken at different temperatures.

TABLE I

DIFFERENCE IN SIZE OF FLARE AT DIFFERENT TEMPERATURES

TEMPERATURE OF AIR (° C.)	SKIN TEMPERATURE (° C.) REPRESENTED BY THERMOCOUPLE NO.				AREA (SQ. CM.) OF FLARE OBTAINED AT SITE OF THERMOCOUPLE NO.			
	1	2	3	4	1	2	3	4
Subject 1								
25.5	31.7	31.4	31.2	31.6	10.0	14.3	15.3	21.3
29.9	36.1	36.0	35.7	35.9	16.9	24.0	28.3	32.8
19.0	30.2	30.3	28.4	28.8	3.1	11.0	14.4	15.7
Subject 2								
29.8	35.2	35.3	35.2	35.2	14.0	24.6	54.0	55.4
25.6	32.3	32.9	32.1	32.3	17.7	21.7	23.4	36.0
20.4	31.1	31.3	31.3	31.9	15.4	17.3	22.1	29.6
Subject 3								
28.2	34.1	34.2	33.6	34.3	---	---	---	23.6
25.1	32.3	31.8	32.3	32.5	---	---	---	14.0
20.2	28.8	29.0	28.7	29.7	---	---	---	10.8

The flares taken in the hot room (86° F.) were considerably larger and the color was a deeper red, and the margins were more irregular. The flares in the cold room (68° F.) were the smallest and palest, and those in the warm room (78° F.) were intermediate both as to size and intensity of color. One of these individuals, Subject 2, showed the most intense flaring I have seen with my dilutions. The area of the flare (1:100,000) in the room at 86° F. was almost twice that obtained in the room at 68° F. Another of the three subjects, Subject 3, reacted only to the strongest solution in all the environmental temperatures used. Subject 1 was about a "normal" reactor. It is interesting to note the slight change of skin temperature that can produce a great change in area of flare.

For all experiments subsequently carried out, a constant temperature room at 78° F. and 40 per cent relative humidity was used.

Since it has been shown that the skin temperatures of a limb depend in part on the position of the limb (Sheard and co-workers[30]), all patients were tested each time in the supine position with arms at a straight angle.

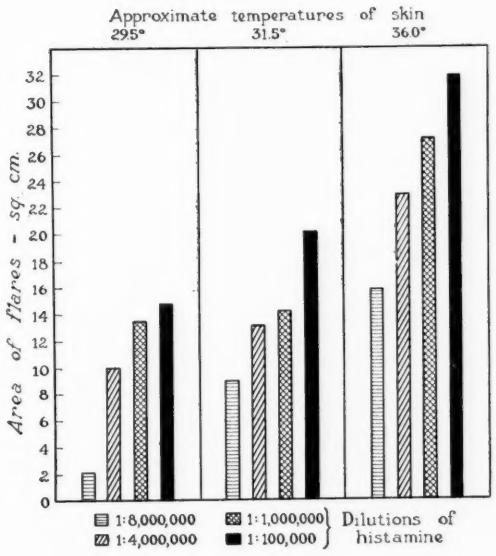

Fig. 1.

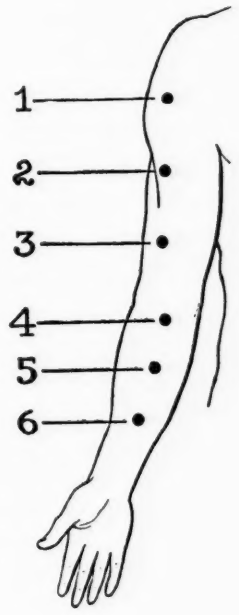

Fig. 2.

Fig. 1.—Changes in the size of flares in the skin of a control volunteer subject at different cutaneous temperatures.

Fig. 2.—Various sites of intradermal injections.

It was decided to use the forearm and upper arm for the sites of injection. It is known that the fingers are customarily colder than the forearm and arm. Now the question arose: Is the skin temperature different at various levels on the limb and is there a lessened gradient as we approach the digits? To determine this, eight normal individuals were placed in the constant temperature room (78° F.) in a supine position and thermocouples were applied to areas subsequently to be used as injection sites; namely, three areas on the volar surface of the forearm and three on the lateral surface of the arm (Fig. 2). Temperature readings were taken every two to three minutes until constant, then the figures were charted and comparisons made. The results are shown in Table II. There were no significant differences in temperature between each of these areas; the average maximal variation of all subjects tested was 0.95° C. in the regions tested. There was no lessened gradient of temperature from No. 1 thermocouple to No. 6. These areas, then, from this standpoint, could be used satisfactorily for the sites of injection and correct comparisons made.

TABLE II

SKIN TEMPERATURE AT VARIOUS SITES ON THE ARM AND FOREARM

PATIENT	ROOM TEMPERATURE (° C.)	TEMPERATURE OF THERMOCOUPLE NO.						MAXIMAL VARIATION (° C.)
		1	2	3	4	5	6	
W. J.	24.9	31.9	31.6	32.0	32.7	32.7	32.0	1.1
L. H.	25.2	32.8	31.2	32.7	32.7	31.4	31.9	1.6
J. V.	25.3	33.8	33.6	33.4	34.8	34.3	35.0	1.6
A. S.	26.2	32.4	32.0	32.0	32.0	32.3	32.0	0.4
A. P.	26.0	33.5	33.0	32.6	33.4	35.5	33.2	0.9
G. K.	25.1	34.8	34.8	---	34.0	34.1	---	0.8
L. P.	25.5	31.7	31.4	---	31.2	31.6	---	0.5
B. M.	25.1	32.5	32.3	---	31.8	32.3	---	0.7
Average variation								0.95

The next step was to decide what dilutions to use. Carrier,[6] Caldwell and Mayo,[16] King,[14] Starr,[31] and DeTakats (and co-workers[32]) used a 1:1,000 dilution of histamine with the cutaneous puncture method, placing a drop of the solution on the skin and puncturing through it. This method is unsatisfactory for a quantitative experiment, for the amount of solution actually introduced is variable as are the size and depth of the puncture wound. Neill and associates[33] and Alexander and co-workers[34] employed known quantities of dilutions varying from 1:2,000 to 1:10,000,000 injected intradermally with a syringe. After trying various dilutions I decided to use four different ones: 1:100,000, 1:1,000,000, 1:4,000,000 and 1:8,000,000. All dilutions were made with normal saline. The reason for employing all of these was that I thought the desensitization process might be a partial or relative thing which would be brought out by some dilutions and not by others. These dilutions in most of the individuals tested produced reactions distinguishable from control injections of the same amount of normal saline. The amount used in each case was 0.05 c.c. The solutions were kept in small, brown glass bottles with rubber diaphragm stoppers. The strength of all solutions was calculated in terms of histamine base. The salt used in each case was histamine acid phosphate. Sharp, 25-gauge hypodermic needles were used and attached to new tuberculin syringes with tight-fitting blue plungers. Each needle was previously cleansed with distilled water, alcohol, and ether, and each syringe with distilled water, and sterilized in dry heat. Injections were made with the least possible trauma into the superficial layers of skin of the volar surface of the forearm and the lateral surface of the arm, approximating the areas used in previous determinations of skin temperatures. Injections were performed by the same individual each time, so the technique was approximately the same. It was found that the flare reached its zenith in about ten minutes and remained the same for about five minutes longer, so the readings were taken ten to fifteen minutes after injection. This eliminates the traumatic

reaction which Neill and co-workers[33] found disappeared within ten minutes. I found (contrary to King) that readings were more easily obtained under artificial light than daylight; therefore, a 75-watt Mazda lamp was placed about two and one-half feet directly over the area for each reading. The method for graphically transferring the flare zone was as follows: When the flares and wheals were well developed, they were outlined on the skin with washable ink. Then thin tissue paper was placed over them and they were traced in pencil onto this and kept for planimeter calculations. Several observers have noted difficulty in determining the border of the flares in dark-skinned and suntanned individuals. This difficulty we did not find serious—there were no Negroes in our series, and the tests were done at a time when sun tan was not prevalent. However, it is true that the intensity and sharpness of the boundary of the flares vary in different individuals. Most of my patients exhibited clear-cut flare margins and in some where I was in doubt as to the exact periphery, looking through a bluish gelatine photographer's filter sometimes made the line of demarcation more distinct. Sites of injection that bled or oozed a relatively large amount of fluid when the needle was withdrawn were not used; the injections were repeated in a neighboring area. Skin testing was done before and after the course of subcutaneous injections and, in most cases, at intervals of three to ten days throughout the treatment. The usual interval between skin tests was three days. When all tests were done, the areas of the flares and wheals were calculated with a Keuffel and Esser planimeter set for square centimeters. The areas of the wheals were disregarded in subsequent procedures.

In order to know whether normal individuals who were not receiving histamine injections subcutaneously exhibited variations in size and intensity of flares over a corresponding period, a control series of skin tests was done in sixteen normal persons. This series of normal individuals consisted of student nurses who were physically fit.

IV. RESULTS

The areas of the flares obtained both in normal control subjects and patients receiving histamine treatment are shown in individual tables (Tables V to LII). In addition, Table III shows the average area of flares over the two-week period in the sixteen normal subjects. It will be seen from this that there is definitely no decrease in area of flares in the normal subjects who did not receive histamine therapeutically over the two-week period but rather a tendency toward an increase. The flares produced by the strongest dilution (1:100,000) were the least variable and showed very little difference in size at each injection. There was a greater change from time to time with the weaker dilutions. For this reason, in further analysis of the figures, the 1:100,000

TABLE III

AVERAGE AREAS OF FLARES OBTAINED WITH DIFFERENT DILUTIONS IN SIXTEEN NORMAL CONTROL SUBJECTS OVER A PERIOD OF TWO WEEKS

DILUTION USED	AVERAGE AREA OF FLARES (SQ. CM.) OVER PERIOD OF TWO WEEKS			
	FIRST INJECTION	SECOND INJECTION	THIRD INJECTION	FOURTH INJECTION
1: 100,000	18.1	19.4	21.8	21.3
1:1,000,000	8.1	9.7	13.6	12.9
1:4,000,000	5.5	7.6	9.3	9.8
1:8,000,000	3.6	5.6	6.6	7.6

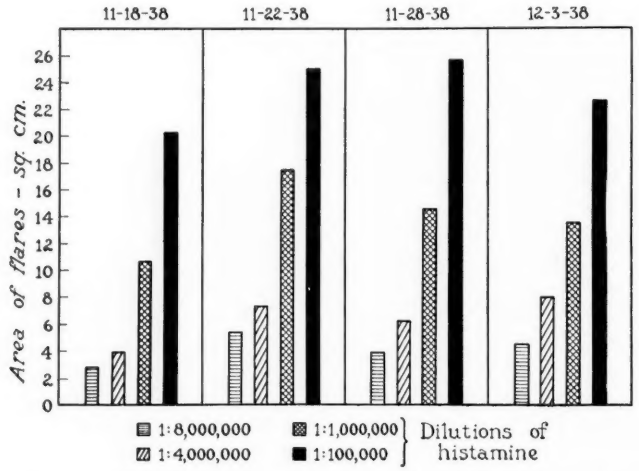

Fig. 3.—Area of flares in a control volunteer subject during a two-week period.

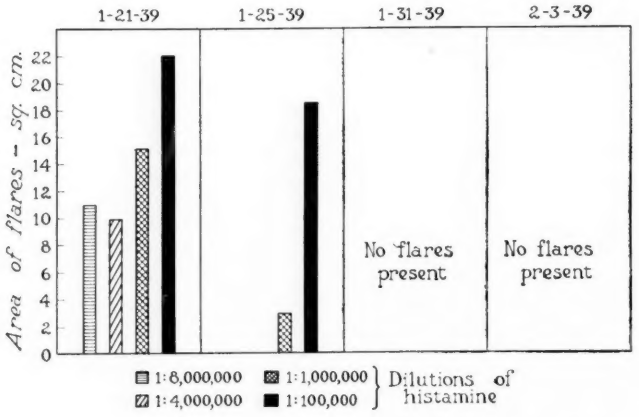

Fig. 4.—Absence of flares in a volunteer subject who was receiving histamine therapeutically.

dilution alone is considered. Of the thirty-two patients who received the histamine treatment, all but two showed a decrease in size of flare at the termination of treatment; thirteen of these, or 40 per cent,

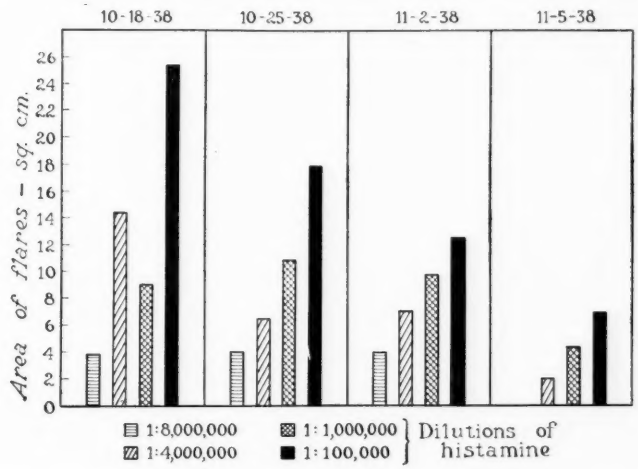

Fig. 5.—Diminution in the size of flares in a subject who was receiving histamine therapeutically.

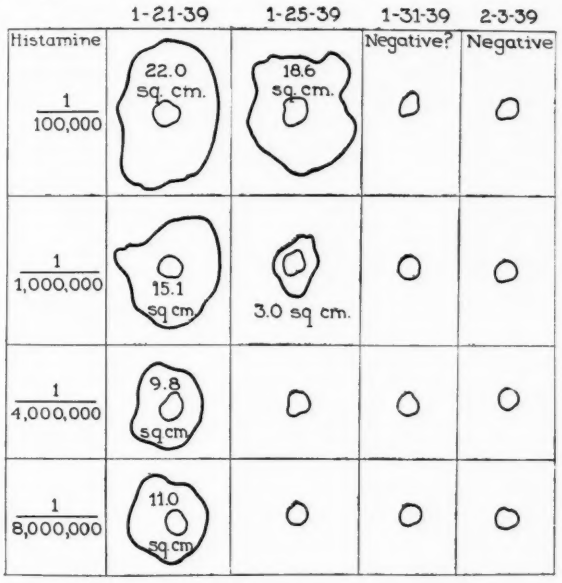

Fig. 6.—Actual tracings (reduced) of flares in a treated volunteer subject.

showed an absence of flare. Since there is bound to be a considerable factor of error in the various procedures from the intradermal injections to the planimeter calculations, despite the most careful technique, it was deemed necessary to determine how much of a reduction in flare size was actually significant and how much could be expected to occur

TABLE IV

CASE	DIAGNOSIS	INITIAL SKIN TEST (SQ. CM.)	FINAL SKIN TEST (SQ. CM.)	CLINICAL RESULT AT END OF TREATMENT
1	Headache	16.1 red	12.2 red	Excellent
2	Headache	28.9 red	Absent	Excellent
3	Headache	10.0 very red	8.3 faint red; other dilutions negative	Excellent
4	Headache	26.5 very red	10.8 faint red	Excellent
5	Hyperhidrosis	14.3 red	15.8 red	No change
6	Drug sensitivity	18.0 red	12.4 red	No change
7	V. M. R.	25.3 very red	6.9 red	Fair
8	Headache	17.2 red	5.6 red	No change
9	Headache	22.9 very red	Absent	Good
10	Urticaria	24.6 red	23.0 red	No change
11	Headache	13.8 red	16.8 faint red	Fair
12	Headache	26.6 red	Absent	Good
13	V. M. R.	14.0 red	7.1 faint red	Excellent
14	Headache	21.8 red	Absent	Fair
15	Headache	16.6 red	Absent	Excellent
16	Headache	31.5 very red	12.0 red	Good
17	Headache	14.5 red	Absent	Excellent
18	Headache	33.9 very red	12.0 red	Fair
19	Headache	24.3 very red	15.6 red	Fair
20	Headache	Initial negative; 4 da. later 19.1, faint	Absent	Good
21	Headache	15.0 faint	Absent	Excellent
22	V. M. R. and headache	Absent	Absent	Excellent
23	Headache	26.8 red	Absent	Fair
24	Urticaria	34.3 very red	18.0 faint red	No change
25	Headache	32.4 red	24.5 red	Fair
26	Hyperhidrosis	21.7 red	20.8 red	No change

TABLE IV—CONT'D

CASE	DIAGNOSIS	INITIAL SKIN TEST (SQ. CM.)	FINAL SKIN TEST (SQ. CM.)	CLINICAL RESULT AT END OF TREATMENT
27	Headache	18.2 red	16.0 faint red	Good
28	Headache	22.6 red	17.1 red	Fair
29	Headache	22.0 red	Absent	Fair
30	Headache	30.4 very red	15.8 faint red	Good
31	Headache	14.3 very red	Absent	Good
32	Headache	No initial flare; 3 days later 9.6, faint	Absent	Good

V.M.R., Vasomotor rhinitis.

without significance. To determine this, the following calculations were done. The figure representing the maximal individual variation of flare size in each normal subject between any two flare areas (1:100,000 dilution only) was noted. Every variation was an increase in flare size toward the end of the two-week period. The greatest variation was 10.8 sq. cm. and the least was 2.1 sq. cm. The mean maximal variation was 6.49 sq. cm. The standard deviation, calculated according to Dunn,[35] proved to be 2.22 sq. cm. Therefore, a change in flare area, in order to be significant, would have to be greater than 6.49 sq. cm. + 2.22 sq. cm. In other words, in order to state that a flare had significantly reduced in size, the difference must have been 8.7 sq. cm. or greater. According to this method, eight patients, or 25 per cent, showed significant reductions, ranging from 8.7 to 21.9 sq. cm., the mean being 15.7 sq. cm. Nine patients, or 28 per cent, showed insignificant reductions ranging from 0.9 to 7.9 sq. cm., the mean being 4 sq. cm. Two patients showed increases of 1.5 and 3 sq. cm., respectively, which are insignificant. These results are shown in Table IV, together with the color intensity of the flares at beginning and end of treatment and the clinical diagnosis. The diagnosis "headache" refers to the syndrome of a vasodilating type of pain previously described. Some of these cases were very typical, others not. It will be noted in addition to the surface area change, that there is generally a decrease in color intensity which is sometimes marked. The clinical result is the patient's estimation of improvement or lack of improvement after a period ranging from one week to six months after treatment. Attempting to correlate the flare change with the clinical result, I found that of the thirteen patients whose flares disappeared, five had excellent results (100 per cent improvement), five good (75 per cent improvement), and three fair (50 per cent improvement). Of the eight

patients exhibiting significant reductions, one had an excellent result, two good, three fair, and two were unchanged. Of the eleven patients exhibiting insignificant reductions or increase in flare, three had excellent results, one good, three fair, and four no change. Probably the most significant fact this information yields is that all patients who showed absence of flare derived some benefit from the treatment.

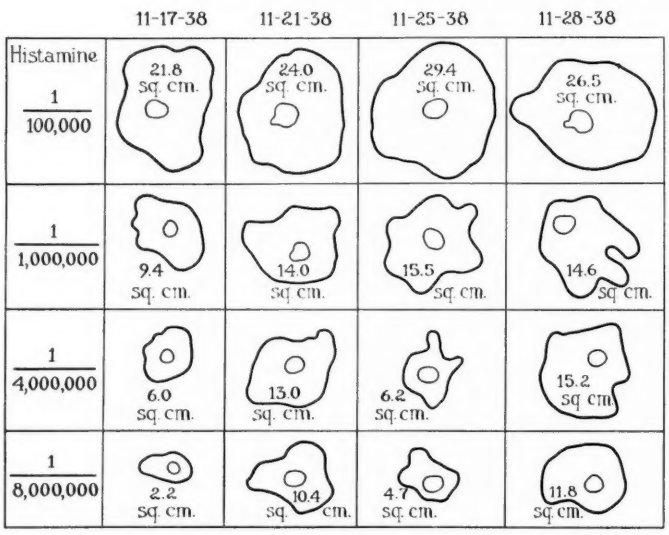

Fig. 7.—Actual tracings (reduced) of flares in a control volunteer subject.

Typical results are graphically represented in Figs. 3, 4, and 5. Fig. 3 shows the sizes of flares over a two-week period in a control subject who did not receive histamine subcutaneously. The relative constancy of the size of the flares (depicted as ordinants) will be noted. Figs. 4 and 5 show, respectively, absence and reduction in the size of the flare in subjects who received histamine therapy. Figs. 6 and 7 are actual tracings of the flare and wheal zones (reduced) in a treated and a control subject, respectively.

V. COMMENT

There are several theoretically possible explanations for the absence and reduction in surface area of the histamine flare after a two-week period of subcutaneous injections of increasing amounts of histamine. That this is not due to the intradermal injections alone is evidenced by the fact that in the controls (who received no histamine therapy) the skin reactions changed insignificantly or increased in size. Therefore, the absence or reduction of flares must have been the result of the repeated, increasing dosages of histamine administered subcutaneously; and we may say that the individuals showing such an absence or reduction exhibited an altered response to histamine at some time during or after the treatment.

What is the mechanism behind the disappearance of the flare? Since the flare is caused by reflex dilatation of arterioles and minute vessels, its disappearance after adequate stimulation can be due either to an interruption in pathways conducting the reflex or to some agent which makes the stimulus inadequate. It is not conceivable to suppose the histamine treatment could in any way interrupt the conducting pathways, so this view may be discarded. Furthermore, such interruption would alter the local sensorium and no such changes were evident in my patients. Then some agent must have been formed as a result of repeated injections of histamine which has a counteracting or neutralizing effect on the amine. There are several possibilities that come to mind here. It is conceivable that there has been an increased production of the histamine-inactivating enzyme, histaminase,[36] as a result of repeated administration of histamine. With an increased amount of histaminase in the body, quantities of histamine previously capable of provoking a response might be rendered inactive. We must assume inactivation was complete within ten minutes (the interval after intradermal injection) when flares were read. This is a short time for histamine inactivation. However, the amount of histamine present in each intradermal injection was so minute that inactivation may have been possible in this length of time. Furthermore, inactivation of histamine within the human organism is not sufficiently well understood to permit me to state that it takes a given period of time to inactivate a given amount of histamine, as can be done in vitro. I attempted to provoke histamine flares in two subjects to whom I had given subcutaneous injections of an histaminase preparation,* 2 c.c. intramuscularly, twice a week for a period of five weeks. I was able to produce normal-sized flares directly after injection of T-360 and at fifteen-minute intervals for two hours after injection. At the termination of the five-week period, histamine still provoked a flare of approximately the same size and intensity. The preparation of histaminase used was not well standardized, and I do not know how much histaminase was actually present. However, the amount that was present had no effect on the production of histamine flares.

It is definitely known that in certain laboratory animals, repeated administration of histamine causes hypertrophy of the adrenal glands. This was observed personally at the Experimental Institute† while I helped Dr. H. E. Essex and Dr. B. T. Horton in work with guinea pigs. It is also an established fact that adrenalin and histamine are to a great extent pharmacologically antagonistic. Perhaps my patients, after repeated stimulation by histamine, were capable of producing more adrenalin and, in this way, counteracting the action of histamine in the skin. I observed one patient to whom adrenalin was given intravenously

*T-360, Winthrop Chemical Co., Inc.

†Of the Mayo Foundation for Medical Education and Research, Rochester, Minn.

after skin tests had been done, and the flares faded very rapidly, whereas they usually persist for about an hour. Recent investigators have shown that the action of adrenalin may be mediated through the potassium-ion.[37] It is known that potassium is definitely connected with skin irritability. Perhaps the failure to obtain the usual histamine flares was due to an increased mobilization of potassium into the skin as a direct or indirect result of previous stimulation by the amine. This would fit in with the work of Lewis and others who found a certain period of unresponsiveness of the skin to urticaria after a severe attack of urticaria. This condition is presumably due to liberation of histamine (or H-substance) into the tissue spaces of the skin.

Perhaps the explanation lies in a combination of these possible factors. It cannot be stated now. In recording my results I mention only the size of the flare and discard the size of the wheal for several reasons. The wheals were usually so small that the error in outlining them, transferring them to paper, and calculating their areas was too great, and furthermore, any change they exhibited was usually insignificant. In only a few cases was there definite reduction in the size of the wheals, and in these they were immense to begin with.

I observed two patients who returned about six weeks after the histamine treatment was done. One of them was having a return of symptoms for which treatment was instituted in the first place. She had previously shown a reduction of flare from 26.5 sq. cm. to 10.8 sq. cm. and on return exhibited a flare of 21.3 sq. cm. The other patient had no return of symptoms. He had previously shown an absence of flare, having originally had one 28.9 sq. cm. On the return visit his flare was 19.2 sq. cm. These results would tend to make one believe that the desensitization process was a short-lived thing, lasting perhaps six weeks or less. I had no further opportunities to investigate this phase.

In three patients receiving histamine treatment I was unable to produce an initial flare with any of the dilutions. One of these patients, E. C., had good flares three days later. They subsequently disappeared and remained absent as long as I observed the patient. This individual had suffered a severe headache of the vasodilating type the day before treatment was started, and it is possible she may have been in an unresponsive or refractory state analogous to that which sometimes follows a severe spontaneous or induced attack of urticaria. Another patient reacted similarly under like conditions. The third patient never exhibited flaring with the dilutions used during the two-week period. He was the only patient in whom I could not produce flaring with the 1:100,000 dilution at any time. The nerve supply to all areas of the skin used for injection was intact.

TABLE V*

CONTROL 1

DILUTION USED	DATE			
	11/18/38	11/22/38	12/3/38	12/9/38
1: 100,000	20.6	14.4	16.7	21.5
1:1,000,000	6.0	8.5	14.0	11.3
1:4,000,000	2.7	5.6	10.9	8.5
1:8,000,000	2.3	3.8	3.5	7.0

*Tables V-LII show the areas of flares in sq. cm. obtained with different dilu'ions of histamine.

TABLE VI

CONTROL 2

DILUTION USED	DATE			
	11/18/38	11/22/38	11/28/38	12/3/38
1: 100,000	20.2	25.0	25.6	22.7
1:1,000,000	10.5	17.5	14.5	13.6
1:4,000,000	3.9	7.3	6.2	8.0
1:8,000,000	2.9	5.4	3.9	4.5

TABLE VII

CONTROL 3

DILUTION USED	DATE			
	11/21/38	12/3/38	12/6/38	12/9/38
1: 100,000	12.9	19.0	20.5	23.7
1:1,000,000	8.9	10.5	11.5	16.5
1:4,000,000	5.7	8.6	10.5	10.0
1:8,000,000	5.4	5.9	6.5	8.0

TABLE VIII

CONTROL 4

DILUTION USED	DATE			
	11/16/38	11/19/38	11/23/38	11/27/38
1: 100,000	14.0	11.6	19.2	16.6
1:1,000,000	3.5	5.2	11.5	8.9
1:4,000,000	2.0	3.9	4.0	3.8
1:8,000,000	2.4	5.9	5.8	1.9

TABLE IX

CONTROL 5

DILUTION USED	DATE			
	11/14/38	11/17/38	11/21/38	11/28/38
1: 100,000	17.0	22.0	19.7	20.9
1:1,000,000	5.5	8.3	12.0	12.0
1:4,000,000	4.7	7.0	6.4	9.2
1:8,000,000	2.0	2.8	3.2	5.5

TABLE X

CONTROL 6

DILUTION USED	DATE			
	11/14/38	11/17/38	11/21/38	11/25/38
1: 100,000	19.1	17.0	18.9	16.8
1:1,000,000	6.6	5.9	8.8	8.8
1:4,000,000	4.0	3.8	6.0	11.7
1:8,000,000	2.6	3.2	5.8	3.9

TABLE XI

CONTROL 7

DILUTION USED	DATE			
	11/15/38	11/18/38	11/22/38	11/26/38
1: 100,000	15.5	17.2	19.4	19.0
1:1,000,000	12.8	6.0	9.0	6.9
1:4,000,000	8.1	4.0	6.6	7.5
1:8,000,000	2.4	3.4	6.8	7.5

TABLE XII

CONTROL 8

DILUTION USED	DATE			
	11/15/38	11/18/38	11/25/38	11/28/38
1: 100,000	14.0	11.8	16.8	13.6
1:1,000,000	6.4	6.0	13.0	11.2
1:4,000,000	5.6	4.9	7.3	6.5
1:8,000,000	4.7	3.1	2.8	5.1

TABLE XIII

CONTROL 9

DILUTION USED	DATE			
	11/17/38	11/21/38	11/25/38	11/28/38
1: 100,000	22.4	25.1	27.0	22.9
1:1,000,000	15.4	11.4	24.0	17.2
1:4,000,000	11.8	9.1	18.0	10.6
1:8,000,000	9.9	4.0	6.2	8.8

TABLE XIV

CONTROL 10

DILUTION USED	DATE			
	11/19/38	12/3/38	12/5/38	12/9/38
1: 100,000	17.8	21.5	23.2	25.7
1:1,000,000	7.1	8.9	14.7	7.0
1:4,000,000	6.4	9.7	9.8	6.9
1:8,000,000	5.0	7.6	8.0	8.8

TABLE XV

CONTROL 11

DILUTION USED	DATE			
	11/14/38	11/17/38	11/21/38	11/26/38
1: 100,000	26.0	22.9	20.3	25.6
1:1,000,000	6.8	10.8	10.3	16.6
1:4,000,000	6.4	10.4	11.6	13.5
1:8,000,000	3.0	8.7	8.2	11.2

TABLE XVI

CONTROL 12

DILUTION USED	DATE			
	11/16/38	11/19/38	12/5/38	12/12/38
1: 100,000	18.7	14.3	25.2	18.0
1:1,000,000	5.2	3.5	13.9	12.5
1:4,000,000	2.8	3.2	6.3	7.0
1:8,000,000	1.9	4.3	7.1	8.3

TABLE XVII

CONTROL 13

DILUTION USED	DATE			
	11/19/38	11/25/38	11/29/38	12/9/38
1: 100,000	13.5	19.8	17.9	23.0
1:1,000,000	7.4	13.7	14.3	18.2
1:4,000,000	7.3	12.0	10.9	14.6
1:8,000,000	2.8	10.0	11.3	10.7

TABLE XVIII

CONTROL 14

DILUTION USED	DATE			
	11/19/38	12/3/38	12/5/38	12/7/38
1: 100,000	17.9	26.1	25.9	27.4
1:1,000,000	8.5	11.0	13.8	16.7
1:4,000,000	5.2	7.6	13.8	13.7
1:8,000,000	6.6	7.2	11.0	10.0

TABLE XIX

CONTROL 15

DILUTION USED	DATE			
	11/18/38	11/22/38	11/28/38	12/7/38
1: 100,000	18.1	18.4	22.8	17.1
1:1,000,000	10.0	14.1	16.2	15.6
1:4,000,000	4.7	12.3	14.3	10.0
1:8,000,000	1.9	3.4	10.1	9.0

TABLE XX

CONTROL 16

DILUTION USED	DATE			
	11/17/38	11/21/38	11/25/38	11/28/38
1: 100,000	21.8	24.0	29.4	26.5
1:1,000,000	9.4	14.0	15.5	14.6
1:4,000,000	6.0	13.0	6.2	15.2
1:8,000,000	2.2	10.4	4.7	11.8

TABLE XXI

HEADACHE (CASE 21)

DILUTION USED	DATE			
	11/24/38	11/28/38	12/2/38	12/8/38
1: 100,000	15.0	10.1	Absent	Absent
1:1,000,000	11.8	Absent		
1:4,000,000	4.6			
1:8,000,000	Absent			

TABLE XXII

URTICARIA (CASE 10)

DILUTION USED	DATE		
	2/10/39	2/16/39	3/1/39
1: 100,000	24.6	14.0	23.0
1:1,000,000	11.2	5.0	11.2
1:4,000,000	6.4	Absent	9.0
1:8,000,000	5.2	Absent	Absent

TABLE XXIII

HEADACHE (CASE 12)

DILUTION USED	DATE	
	2/25/39	3/9/39
1: 100,000	26.6	Absent
1:1,000,000	13.9	
1:4,000,000	Absent	
1:8,000,000		

TABLE XXIV

HEADACHE (CASE 31)

DILUTION USED	DATE		
	10/20/38	10/25/38	10/29/38
1: 100,000	14.3	15.3	Absent
1:1,000,000	4.7	5.8	
1:4,000,000	2.5	2.5	
1:8,000,000	2.3	2.0	

TABLE XXV

VASOMOTOR RHINITIS (CASE 3)

DILUTION USED	DATE		
	12/3/38	12/9/38	12/13/38
1: 100,000	14.0	12.7	7.1
1:1,000,000	9.6	6.9	6.0
1:4,000,000	8.7	4.2	Absent
1:8,000,000	5.2	Absent	

TABLE XXVI

VASOMOTOR RHINITIS (CASE 7)

DILUTION USED	DATE			
	10/18/38	10/25/38	11/2/38	11/5/38
1: 100,000	25.3	17.8	12.4	6.9
1:1,000,000	9.0	10.9	9.7	4.3
1:4,000,000	14.4	6.4	7.0	2.1
1:8,000,000	3.9	4.0	4.0	Absent

TABLE XXVII

HEADACHE (CASE 29)

DILUTION USED	DATE			
	1/21/39	1/25/39	1/31/39	2/3/39
1: 100,000	22.0	18.6	Absent	Absent
1:1,000,000	15.1	3.0		
1:4,000,000	9.8	Absent		
1:8,000,000	11.0			

TABLE XXVIII

HEADACHE (CASE 30)

DILUTION USED	DATE			
	11/25/38	11/30/38	12/9/38	12/16/38
1: 100,000	30.4	21.7	21.9	15.8
1:1,000,000	13.2	16.1	10.9	13.0
1:4,000,000	11.4	11.0	10.8	9.8
1:8,000,000	10.1	11.1	10.0	10.1

TABLE XXIX

HEADACHE (CASE 11)

DILUTION USED	DATE	
	2/23/39	3/9/39
1: 100,000	13.8	16.8
1:1,000,000	10.5	10.0
1:4,000,000	4.7	4.1
1:8,000,000	Absent	Absent

TABLE XXX

HEADACHE (CASE 9)

DILUTION USED	DATE	
	2/29/39	2/20/39
1: 100,000	22.9	Absent
1:1,000,000	9.3	
1:4,000,000	6.0	
1:8,000,000	3.1	

TABLE XXXI

HEADACHE (CASE 28)

DILUTION USED	DATE			
	1/18/39	1/23/39	1/31/39	2/3/39
1: 100,000	22.6	16.5	9.4	17.1
1:1,000,000	17.6	14.0	Not done	10.9
1:4,000,000	15.0	10.9	Not done	6.9
1:8,000,000	11.9	Absent	Not done	Absent

TABLE XXXII

HEADACHE (CASE 27)

DILUTION USED	DATE		
	1/18/39	1/24/39	1/31/39
1: 100,000	18.2	14.2	16.0
1:1,000,000	16.5	7.0	8.1
1:4,000,000	15.3	5.9	7.1
1:8,000,000	13.6	Absent	Absent

TABLE XXXIII

HYPERHIDROSIS (CASE 26)

DILUTION USED	DATE			
	11/18/38	11/22/38	12/2/38	12/10/38
1: 100,000	21.7	18.0	20.5	20.8
1:1,000,000	7.0	13.9	10.4	11.0
1:4,000,000	6.0	15.8	8.9	10.7
1:8,000,000	2.5	8.0	9.4	4.1

TABLE XXXIV

HEADACHE (CASE 25)

DILUTION USED	DATE		
	12/6/38	12/10/38	12/13/38
1: 100,000	32.4	14.9	24.5
1:1,000,000	14.5	11.4	11.0
1:4,000,000	12.5	9.7	11.0
1:8,000,000	9.1	14.7	8.5

TABLE XXXV

URTICARIA (CASE 24)

DILUTION USED	DATE	
	1/23/39	2/7/39
1: 100,000	34.3	18.0
1:1,000,000	19.4	10.5
1:4,000,000	17.0	Absent
1:8,000,000	13.1	Absent

TABLE XXXVI

VASOMOTOR RHINITIS AND HEADACHE (CASE 22)

DILUTION USED	DATE		
	11/29/38	12/1/38	12/6/38
1: 100,000	Absent	Absent	Absent
1:1,000,000	Absent	Absent	Absent
1:4,000,000	Absent	Absent	Absent
1:8,000,000	Absent	Absent	Absent

TABLE XXXVII

HEADACHE (CASE 20)

DILUTION USED	DATE		
	11/26/38	11/30/38	12/8/38
1: 100,000	Absent	19.1	Absent
1:1,000,000	Absent	14.6	Absent
1:4,000,000	Absent	11.4	Absent
1:8,000,000	Absent	7.4	Absent

TABLE XXXVIII

HEADACHE (CASE 8)

DILUTION USED	DATE			
	11/22/38	11/28/38	12/2/38	12/6/38
1: 100,000	17.2	14.1	11.9	5.6
1:1,000,000	13.5	10.8	11.7	4.9
1:4,000,000	9.7	9.3	7.6	3.0
1:8,000,000	8.5	7.7	6.5	Absent

TABLE XXXIX

DRUG SENSITIVITY (CASE 6)

DILUTION USED	DATE			
	11/29/38	12/2/38	12/5/38	12/12/38
1: 100,000	18.0	13.3	14.1	12.4
1:1,000,000	13.1	12.8	9.8	6.5
1:4,000,000	8.0	7.1	8.0	Absent
1:8,000,000	9.0	5.1	7.0	Absent

TABLE XXXX

HYPERHIDROSIS (CASE 5)

DILUTION USED	DATE				
	11/16/38	11/19/38	11/23/38	11/26/38	12/5/38
1: 100,000	14.3	15.6	6.9	13.0	15.8
1:1,000,000	7.5	4.3	6.3	9.8	3.6
1:4,000,000	7.2	3.9	6.7	Absent	2.8
1:8,000,000	1.6	2.4	5.9	Absent	2.0

TABLE XXXXI

HEADACHE (CASE 4)

DILUTION USED	DATE					
	10/26/38	10/29/38	11/2/38	11/4/38	11/11/38	11/21/38
1: 100,000	26.5	19.8	12.3	14.1	15.9	10.8
1:1,000,000	26.3	9.3	9.7	10.8	14.8	5.5
1:4,000,000	14.4	7.5	5.4	2.9	6.0	5.0
1:8,000,000	5.5	3.0	2.7	2.4	4.2	Absent

TABLE XXXXII

HEADACHE (CASE 3)

DILUTION USED	DATE			
	11/4/38	11/8/38	11/11/38	11/18/38
1: 100,000	10.0	11.3	9.0	8.3
1:1,000,000	9.3	6.2	1.9	Absent
1:4,000,000	4.0	5.3	1.9	
1:8,000,000	6.0	Absent	Absent	

TABLE XXXXIII

HEADACHE (CASE 14)

DILUTION USED	DATE			
	1/13/39	1/20/39	1/23/39	1/31/39
1: 100,000	21.8	6.0	Absent	Absent
1:1,000,000	13.8	Absent		
1:4,000,000	6.8			
1:8,000,000	Absent			

TABLE XXXXIV

HEADACHE (CASE 2)

DILUTION USED	DATE		
	11/11/38	11/15/38	11/19/38
1: 100,000	28.9	Absent	Absent
1:1,000,000	11.0		
1:4,000,000	9.2		
1:8,000,000	5.6		

TABLE XXXXV

HEADACHE (CASE 1)

DILUTION USED	DATE		
	10/25/38	11/1/38	11/7/38
1: 100,000	16.1	12.9	12.2
1:1,000,000	4.4	4.0	3.9
1:4,000,000	3.3	3.7	3.3
1:8,000,000	3.2	2.5	4.4

TABLE XXXXVI

HEADACHE (CASE 32)

DILUTION USED	DATE			
	11/2/38	11/5/38	11/9/38	11/14/38
1: 100,000	Absent	9.6	4.2	Absent
1:1,000,000		7.5	Absent	
1:4,000,000		4.6		
1:8,000,000		5.0		

TABLE XXXXVII

HEADACHE (CASE 19)

DILUTION USED	DATE		
	10/10/38	10/20/38	10/24/38
1: 100,000	24.3	22.5	15.6
1:1,000,000	16.4	7.4	5.0
1:4,000,000	6.3	2.5	5.3
1:8,000,000	4.8	3.2	Absent

TABLE XXXXVIII

HEADACHE (CASE 18)

DILUTION USED	DATE	
	10/22/38	10/28/38
1: 100,000	33.9	12.0
1:1,000,000	31.0	Absent
1:4,000,000	13.5	
1:8,000,000	8.0	

TABLE XXXXIX

HEADACHE (CASE 17)

DILUTION USED	DATE		
	11/4/38	11/9/38	11/14/38
1: 100,000	14.5	7.6	Absent
1:1,000,000	10.5	5.0	
1:4,000,000	6.0	7.3	
1:8,000,000	3.5	3.7	

TABLE L

HEADACHE (CASE 16)

DILUTION USED	DATE				
	10/26/38	10/29/38	11/1/39	11/4/38	11/7/38
1: 100,000	31.5	21.4	19.3	20.1	12.0
1:1,000,000	28.0	13.7	9.4	13.0	8.9
1:4,000,000	8.7	5.9	3.9	8.8	7.4
1:8,000,000	5.9	Absent	Absent	8.2	3.9

TABLE LI

HEADACHE (CASE 15)

DILUTION USED	DATE		
	1/12/39	1/18/39	1/25/39
1: 100,000	15.6	11.8	Absent
1:1,000,000	11.2	Absent	
1:4,000,000	7.0		
1:8,000,000	Absent		

TABLE LII

HEADACHE (CASE 23)

DILUTION USED	DATE		
	12/13/38	12/17/38	12/19/38
1: 100,000	26.8	Absent	Absent
1:1,000,000	18.1		
1:4,000,000	14.0		
1:8,000,000	8.8		

VI. CONCLUSIONS

1. The volar surface of the forearm and lateral surface of the arm can be used satisfactorily for comparative studies of histamine skin reactions.

2. To evaluate histamine flares correctly, they must be produced under carefully controlled conditions: constant temperature environment, standard posture of the individual and the arm to be tested, standard source of light, and uniformity of technique. I found a room at 78° F. and 40 per cent humidity, and the supine position of the patient with arm at straight angle satisfactory, and a 75-watt Mazda lamp placed two and one-half feet directly over the flare zone preferable to daylight for the reading of flares.

3. The histamine flare varies in size and color intensity directly with the skin and environmental temperature. Since many skin reactions are dependent on the release of histamine or histamine-like substance from the skin, this fact probably holds true for a wide variety of other skin reactions as well and should be borne in mind when comparative studies are made. Only a slight change in skin temperature apparently is necessary to produce a great change in the area of the flare.

4. The most useful dilution of histamine for skin testing of those I tried is 1:100,000 (histamine acid phosphate used, dilution calculated as base).

5. The histamine wheal is not a good criterion to use in comparative studies of endermic reactions.

6. Histamine flares provoked at intervals over a period of approximately two weeks in normal subjects show no significant variation in size or color, or, if they do, perhaps a slight increase in size toward the end of the period.

7. Histamine flares provoked at intervals over a period of approximately two weeks in subjects treated with gradually increasing amounts of histamine administered subcutaneously twice daily for the same period disappear in 40 per cent of cases, are significantly reduced in 25 per cent, are insignificantly changed in 35 per cent. There is an apparent correlation between clinical result of treatment and state of histamine flare. If we assume the skin to be an index to systemic desensitization (as it is in allergic states), we may say that 40 per cent of the patients treated showed a complete desensitization, 25 per cent showed partial desensitization, and 35 per cent showed no desensitization. The desensitization phenomenon is probably accompanied by much individual variation and is perhaps relatively short-lived.

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THE NEWER INTERPRETATION OF POSITIVE SKIN REACTIONS

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THE use of skin tests is a routine procedure in the practice of allergy. The understanding of their interpretation is of the utmost importance both in diagnosis and treatment. But despite their extensive use and admitted consequence, very little practical information concerning their interpretation has appeared in the literature. Allergy texts contain such statements as "A positive specific reaction . . . means simply that the skin is specifically sensitive . . . it does not necessarily imply that this patient is likewise clinically sensitive to this allergen or that it is of importance as an etiologic factor."[1] Another statement frequently made is that the interpretation of positive skin reactions should be considered tentative until confirmation is secured by (1) correlation with the patient's history, (2) by deliberate exposure of the patient to the allergen, or (3) by treatment. This assertion implies that the confirmatory tests have greater diagnostic value than do the specific skin reactions.

The correlation of the positive skin reaction with the patient's history is possible in only a small proportion of cases. Thus, a positive skin reaction to ragweed pollen in a patient with a clinical history of fall hay fever may be considered conclusive evidence that the ragweed pollen is the etiologic factor. However, such a correlation eliminates the possibility of extra pollen factors playing any role whatsoever in the production of the symptoms. In the largest proportion of cases, particularly when ubiquitous substances are involved, such as foods and house dust, such a correlation is impossible from a practical standpoint. Nonspecific factors, such as weather and infections, on the one hand and clinical sensitivity to multiple substances on the other, prevent an accurate correlation.

Deliberate exposure of the patient to the substance or the removal of the substance from the patient does not necessarily offer conclusive evidence of the allergic nature of the patient's symptoms. Neither the induction of symptoms following the ingestion nor their absence following the removal of foods in patients with suspected gastrointestinal allergy necessarily implies that the symptoms are the results of an allergic reaction. Foods can and do produce dyspeptic symptoms by other mechanisms. The patient with peptic ulcer, or chronic cholecystitis, or

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spastic colitis develops dyspepsia as the result of the ingestion of certain foods. The origin of the symptoms in these instances is clearly not allergic in nature. The development of symptoms following inhalation of a substance also does not prove the allergic basis of the symptoms. It is not possible to distinguish between the irritant and the allergic reaction to house dust on such exposure. Furthermore, many substances, such as molds and feathers, contain large amounts of irritant substances, and with these, also, it is not possible to distinguish between the allergic and the nonallergic reaction.

Failure to induce symptoms by exposure of a patient to a substance does not rule out the possibility that the particular substance is clinically important as an etiologic factor. Such tests frequently do not duplicate or even approximate clinical conditions. The dose of allergen to which a patient is exposed in these tests is usually large and the time of exposure is usually short, whereas under clinical conditions prolonged exposure and relatively small dosage are the rule. Furthermore, the nonspecific environment, such as the meteorologic and psychologic milieu can rarely, if ever, be duplicated.

The effect of therapy cannot be considered as conclusive evidence for or against specific clinical sensitization. Reasoning from the results of therapy, whether successful or unsuccessful, is likely to result in the logical fallacy of "*post hoc, ergo propter hoc.*" Clinical improvement may often be due to agents other than the allergic factor. Although this observation has been made many times, it was recently demonstrated clearly by Loveless and Baldwin's studies on "coli metabolin" therapy of ragweed hay fever.[2] These investigators found that there was no statistically significant difference in the incidence of improvement of symptoms when either "coli metabolin" or physiologic salt solution was used as the therapeutic agent, but that the incidence of improvement was significantly greater when either solution was used therapeutically than when no treatment at all was given. It was therefore evident that improvement of hay fever symptoms can be accomplished by psychologic means. We cannot, however, reason that, as the result of this study, the etiology of hay fever is psychogenic in origin. Failure to ameliorate symptoms by means of specific therapy may have no bearing whatsoever on the etiologic importance of a particular substance. All too many patients who are clinically sensitive to specific pollens fail to obtain therapeutic benefit from specific therapy.

It is therefore quite evident that the recommended confirmatory tests are inconclusive. None of these tests are critical of clinical sensitivity. They are merely additional presumptive or circumstantial evidence of specific sensitivity. Whether or not the confirmatory tests have greater value as diagnostic criteria of clinical sensitivity than do specific skin reactions remains to be determined.

The specific or allergic skin reaction is the expression of the reaction between the specific antibody, reagin, and its corresponding allergen. The reagin-allergen reaction presumably produces the release of a histamine-like substance, or perhaps of histamine, which acts upon the skin to produce the wheal and the surrounding erythema. Whealing, however, is a fundamental form of skin response which may be produced by many mechanisms, the allergic reaction being but one which can bring forth this response. Consequently skin reactions are of two types: (1) specific, wheal responses which are produced by the allergic mechanism; and (2) nonspecific, wheal responses which are produced by mechanisms other than allergic.

The nonspecific reactions may be produced by the following: (1) trauma; (2) primary urticariogenic substances, such as morphine, codeine, atropine, pilocarpine;[3] and (3) allergenic extracts which have nonspecific properties because of their concentration, or their glycerin or histamine content.

Trauma can produce the wheal response even in normal skin. It is, of course, a much more important factor in skin which has a dermographic tendency. Trauma is undoubtedly responsible for the majority of wheal and flare reactions to the intradermal injection of physiologic saline solution. A small but definite proportion of patients react with wheal and erythema when 0.01 c.c. of physiologic salt solution is injected intracutaneously. A smaller proportion of patients show this reaction with physiologic saline solution when the scratch test is performed.

Glycerin is also capable of producing nonspecific reactions. With intracutaneous tests, concentrations of glycerin in physiologic salt solution above 5 per cent produce a significantly greater incidence of wheal and flare reactions than does physiologic salt solution. The incidence of such reactions produced by concentrations of 5 per cent or less of glycerin in physiologic saline solution is not significantly greater than that produced by such salt solution alone.

All extracts, whether of house dust, pollen, inhalants, molds, or foods, can be made to produce nonspecific skin reactions by sufficient increase in their concentration. As usually prepared, only a few allergenic extracts will produce a high incidence of such reactions with the scratch test. On the other hand, with intracutaneous tests it is necessary to dilute the extracts in order to avoid such reactions.

Histamine, of course, produces a nonspecific reaction. The effect of histamine is much more apparent with the intracutaneous test than with the scratch test.

Since skin reactions to extracts may or may not be on an allergic basis, it is necessary to distinguish between specific and nonspecific reactions. This distinction is accomplished by means of a biologic

method. We determine the incidence of positive reactions which a particular extract produces in a control series (individuals who have no symptoms attributable to allergic disease). We also determine the incidence of positive reactions which this particular extract produces in a series of patients whose allergic symptoms are attributable to the particular substance in question. We then compute whether these incidences of positive reactions are statistically significant. If we find that the particular extract produced significantly fewer positive reactions in the control series than in the series of patients who are apparently allergic to the particular substance in question, then the reactions which we obtained, in both the control and the patient series, were specific. On the other hand, if there was no significant difference in the incidence of positive reactions in the two series, the particular extract under investigation was irritant, and we can make no evaluation concerning the allergic nature of any proportion of these reactions.

Two examples will illustrate this:

In Example I a 0.075 solution of purified house dust extract gave fifteen positive scratch test reactions in 200 control patients and 125 positive reactions in 160 patients with perennial hay fever and asthma (Table I).[4,5]

TABLE I

	POSITIVE	NEGATIVE	TOTAL	PER CENT POSITIVE
Control patients	15	185	200	7.5
Hay fever and asthma patients	125	35	160	78.1

The difference in the incidence of the positive reactions in the two series is statistically significant. (The computation may be made by either the Chi square test or by the difference between two proportions.) We therefore conclude that the positive reactions are specific.

Example II is in contrast to Example I. A house dust extract was reported as producing twenty positive reactions in thirty control patients and sixty-five positive reactions in eighty allergic patients[6] (Table II).

TABLE II

	POSITIVE	NEGATIVE	TOTAL	PER CENT POSITIVE
Control patients	20	10	30	66.7
Asthma and hay fever patients	65	15	80	81.3

The difference in incidence of the positive reactions in the two series of patients is statistically not significant. Therefore, this house dust extract produced nonspecific reactions, and no evaluation can be made concerning their allergic nature.

I wish to emphasize the fact that specificity or nonspecificity can be determined only by biologic methods. Specificity and nonspecificity cannot be designated or determined by the computation of physical or chemical "units" in extracts. The statement that an extract contains 1,000 "protein or nitrogen units" or is a 1:5,000 solution does not indicate its biologic ability to produce skin reactions. This can be ascertained only by actual skin testing. If such an extract is used without determination of the property to produce skin reactions, the user assumes that this particular extract will produce specific skin reactions. He has, however, very little justification for such an assumption. This is particularly true when the intracutaneous test is used. The specificity of skin reactions can be determined only by the relatively simple comparative biologic method described above. There is no need for guesses and assumptions.

The specific skin reaction is due to the interaction in the skin between the allergen and its specific antibody, reagin. When other shock organs are sensitized to a particular allergen, specific reagin is also present in them, and the interaction of allergen and reagin presumably is responsible for allergic symptoms. However, this mechanism alone does not explain the development of allergic disease. Black[7] first suggested that the reagin-allergen reaction was not the cause of clinical symptoms. Another and as yet undetermined mechanism must function to precipitate the symptoms of allergic disease. That a mechanism other than the reagin-allergen reaction is concerned with the precipitation of allergic symptoms is evidenced by the following:

1. It is common experience that the incidence of specific skin sensitivity is significantly greater than that of clinical sensitivity. This is manifested not only by the incidence of specific sensitivity in individuals who have no symptoms attributable to allergic disease (as the control individuals), but also by the incidence of multiple specific skin sensitivity without clinical symptoms in patients who have allergic disease from one or several substances. An example is the not infrequent finding of skin sensitivity to grass or tree pollens in patients who have allergic symptoms from ragweed only. Another example is the presence of specific sensitivity to pollens in patients whose allergic symptoms are caused only by house dust. This phenomenon is so well known that many authors have termed such specific skin reactions as expressions of either past or potential clinical sensitivity.

2. It has been demonstrated that specific skin sensitivity may antedate clinical sensitivity by quite a long time. For example, Phillips[8] has reported the development of specific sensitivity to sugar beet pollen one year before the onset of clinical symptoms.

3. It is common experience that specific skin sensitivity may remain long after clinical sensitivity has disappeared. Not only is this a fre-

quent experience in routine allergy practice, but it also occurs in cases of cestode infection. In echinococcus infection, the skin reaction is positive not only as long as cysts remain in the patient but may continue so for five to seven years after surgical removal of all cysts.[9]

4. Although specific skin sensitivity is demonstrable whether symptoms are present or not, allergic symptoms are commonly intermittent.

It is, therefore, apparent that specific skin reactions may or may not be relevant to the presenting symptoms. Those that are relevant are the diagnostic reactions which constitute the clinically significant fraction of the total number of specific reactions.

Specific skin reactions occurring in control patients (individuals who have no symptoms attributable to allergic disease) are devoid of etiologic significance with respect to presenting symptoms. It is also apparent that not all of the specific reactions occurring in patients with allergic disease are etiologically relevant to the presenting symptoms. Consequently, such specific reactions must be taken into account when we are estimating the proportion of specific reactions which are diagnostic.

The diagnostic reactions are estimated as the percentage of positive reactions in patients whose symptoms are attributable to the particular substance in question divided by the sum of the per cent of positive reactions in these patients and the per cent of positive reactions in control patients. The data of our first example are contained in Table III.

TABLE III

	POSITIVE	NEGATIVE	TOTAL	PER CENT POSITIVE
Control patients	15	185	200	7.5
Asthma and hay fever patients	125	35	160	78.1

The proportion of these specific reactions that is diagnostic would be

$$\frac{78.1}{78.1 + 7.5} = .912 \text{ or } 91.2\%.$$

This means that about 91 per cent of the specific reactions that were obtained with this extract are diagnostic. That is to say, 91 out of every 100 positive reactions obtained on patients with perennial hay fever and/or asthma with this house dust extract were relevant to the presenting symptoms. Stated in another way, the odds are about 9 to 1 that a positive reaction obtained with this concentration of the extract is etiologically relevant to the presenting symptoms.

Weil[10] has suggested an alternate method of estimating the diagnostic reactions. His formula is the percentage of positive reactions in patients whose symptoms are attributable to the particular substance in question minus the percentage of positive reactions in control patients divided by the percentage of positive reactions in patients whose symptoms are attributable to the particular substance in question.

According to Weil's modification, the calculation of the diagnostic reactions in the above example is

$$\frac{78.1 - 7.5}{78.1} = 90.4\%.$$

SUMMARY

1. Positive skin reactions may be specific or nonspecific.

2. Specific reactions are the expression of the reaction between reagin and its corresponding allergen. They are distinguished from nonspecific reactions by means of a comparative biologic method which utilizes statistical tests of significance.

3. The reagin-allergen reaction alone does not explain the development of allergic symptoms, hence only a fraction of the specific reactions are relevant to the presenting symptoms.

4. Two formulas are presented for the estimation of the diagnostic reactions produced by a given extract.

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SPONTANEOUS AND INDUCED SENSITIVITY TO FOODSTUFFS: X-RAY STUDIES OF THE SMALL INTESTINE IN MAN AND GUINEA PIG

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ROENTGENOLOGIC studies of the allergic gastrointestinal tract have been carried out by several investigators as a means of studying the altered physiology as well as an attempt to formulate a diagnostic tool. The clinical allergist is well aware of the difficulty in basing a diagnosis of food allergy on the cutaneous test. In the presence of a negative skin reaction, associated gastrointestinal allergic phenomena, after ingestion of a suspected food, led to a search for evidence in gastrointestinal visualization. The local reaction of the sensitized wall of the alimentary canal has been observed directly and the histopathology studied by Gray, Walzer, and Grayzel[1, 2, 3, 4] and their co-workers.

Fries and Zizmor[5] in their study of a group of children with gastrointestinal allergy directed their attention to alteration in the emptying time of the stomach, to pylorospasm, and to the tone of the colon. They mentioned dilated loops of jejunum in two of their patients; another patient showed a somewhat peculiar small bowel pattern.

In the radiologic study of the alimentary canal in sprue, Mackie,[6] in 1933, called attention to abnormal changes in the pattern of the small intestine. These observations were extended by Snell and Camp,[7] Mackie and Pound,[8] and Mackie, Miller and Rhoads.[9] The variations have been grouped together under the term deficiency state since the first observations were made in cases of sprue, chronic steatorrhea, and severe chronic ulcerative colitis, showing vitamin deficiencies. The deficiency state pattern is not, however, peculiar to vitamin deficiencies but has been found in other diseases including nephrosis,[10] diabetes insipidus,[10] obstructive jaundice,[11] and mesenteric lymphatic obstruction. Golden[12] reviewed the literature and described the syndrome of the deficiency state variable as it relates to the small intestine but he stated that it was possible to demonstrate (1) alteration of normal motility, with hypermotility in the early and hypomotility in the late cases, (2) hypertonicity, (3) dilatation, (4) abnormal segmentation suggesting spasm, (5) coarsening of the mucosal folds, and (6) flocculation of the barium shadow. The sum of the changes in varying degrees is re-

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ferred to as the pattern of the deficiency state. Most of these patients have shown a reversal toward the normal pattern following adequate treatment.

In a case of the delayed type of food allergy reaction reported by Hampton and Cooke,[13] there was noted abnormal segmentation of the small intestine following a barium meal containing the allergen. This observation stimulated a search for other cases of intolerance for specific foods to determine whether or not such abnormalities of the pattern of the small intestine were a common finding or resembled those seen in the deficiency state. Patients were chosen for study who had a clinical history of intolerance for a specific food, or in whom a markedly positive skin test called attention to a specific food as the possible cause of allergic symptoms. Attempts were made to provoke symptoms by the addition of the foods to the diet after a period of abstinence from them and were paralleled by roentgenographic study of the response to the implicated food.

CLINICAL STUDY

In the present series, nine cases of gastrointestinal allergy were studied with particular reference to the behavior of the small intestine, both with and without the addition of the suspected food to the barium meal. Certain subjects were given the food in the barium at the time of the examination while others were allowed to ingest the food at certain intervals before the examination. The time for ingestion of the suspected food was selected on the basis of the previous history in order to outline the small intestine at the time of the most severe symptoms. Barium sulfate, containing a small amount of carbohydrate to improve the suspension, was employed. Immediate films of the stomach and upper small bowel were made, followed by two-, four-, and six-hour films of the small intestine. A twenty-four-hour film of the barium-filled colon was also made. In most cases the patient was aware of the type of meal offered, although in several the allergic meal preceded the control meal without the patient's knowledge.

Of nine patients suspected of having gastrointestinal allergy because of clinical intolerance for specific foods, seven showed distinct variation of the small bowel pattern between the control series and that containing the suspected food. In four of these the difference was not enough to exclude the possibility that the changes were due to the nutritive value of the meal (Fig. 1). In three, however, the changes were of such a degree that they could not be explained as due to the nutritive food value, but were thought to be the consequence of specific allergic intolerance. In these three cases (E. J., M. M., and C. Z.) the appearance of the small bowel pattern was that of the deficiency state (Fig. 2). The allergic histories, skin tests, and x-ray findings are presented in Table I.

TABLE I

PATIENT SERIES

CASE	AGE (YR.)	SEX	INTRACUTANEOUS TESTS	SMALL INTESTINAL PATTERN	SIGNIFICANT VARIATIONS FROM CONTROL	EXCESSIVE CHANGES (DEFICIENCY PATTERN)
R. F.	44	M.	Orange, ++++	Coarsening of mucosal folds; spasm	Yes	
A. H.	53	F.	Not done	Segmentation; spasm; abnormal mucosal markings	Yes	
P. S.	30	F.	Not done	Spasm; segmentation	Yes	
M. P. R.	8½	F.	Milk whey, 0 Casein, 0 Milk whey proteoses, + Casein proteoses, +	Flocculation; coarsening of mucosal folds	No	
M. R.	27	F.	Milk whey, 0 Casein, 0 Milk whey proteoses, + Casein proteoses, +	Spasm; segmentation	Yes	
E. S.	29	M.	Eggs, 0 Wheat, 0 Proteoses, 0	No alteration in small intestine pattern; definite change in colon with test meal	No	
E. J.	48	F.	Milk, 0	Coarsening of mucosal folds; dilatation; segmentation	Yes	Yes
M. M.	37	M.	Milk, 0 Chocolate, 0 Proteoses chocolate, ±	Segmentation; spasm dilation	Yes	Yes
C. Z.	32	M.	Milk whey, 0 Milk whey proteoses, ++ Casein, 0 Casein proteoses, 0	Segmentation; spasm	Yes	Yes

R. F., Pollen hay fever and asthma for eighteen years; continual asthma for two years; dust and feather treatment did not change this; orange- and citrus fruit-free diet gave relief; symptoms provoked three times by adding food.

A. H., Mild ulcerative colitis, sixteen years; improved for five years on milk-free diet; four trials on milk reproduced a mucous diarrhea.

P. S., Mild colitis; was not studied from point of view of allergy; when given milk enema complained of pain and inability to expel enema; fainted during examination; control barium enema, no reaction.

M. P. R., Daughter of M. R.; no colitis but has cramps after taking milk; on more than three occasions symptoms with milk, but can occasionally take small amounts without symptoms; abdominal cramps during milk series.

M. R., Mother of M. P. R.; minimal colitis; milk-free diet six years with improvement; pain, increase in stools, and melena at time of milk series.

E. S., Under treatment for ragweed and timothy hay fever; migraine headache, watery stools, and tenseness twenty-four to thirty-six hours after egg or wheat meals; symptoms with test series at time of x-ray study.

(Continued at bottom of facing page.)

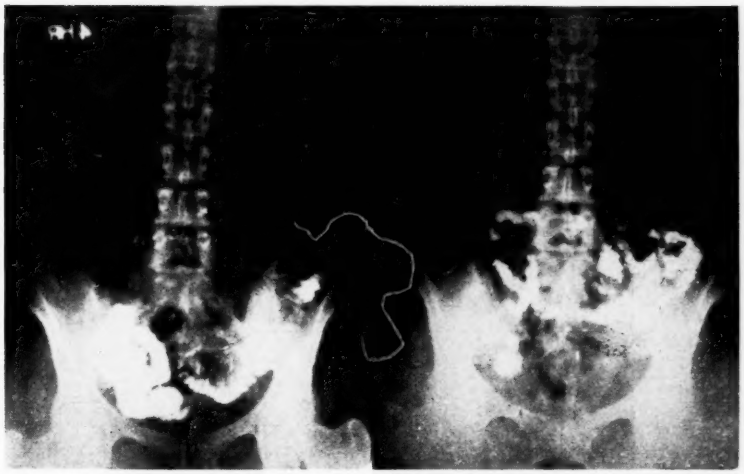

Fig. 1.—Patient A. H. The film on the left represents the control series which shows normal small bowel pattern and approximately normal motility. There is some flocculation of the barium in the upper jejunum, but this appears within normal limits. The mucosal folds seen in the lower half of the abdomen show a typical normal appearance. The film on the right is that following ingestion of the allergic meal. There is segmentation of the barium column, probably the result of spasm. The motility is considerably altered as compared with the control, and no normal valvulae conniventes are outlined.

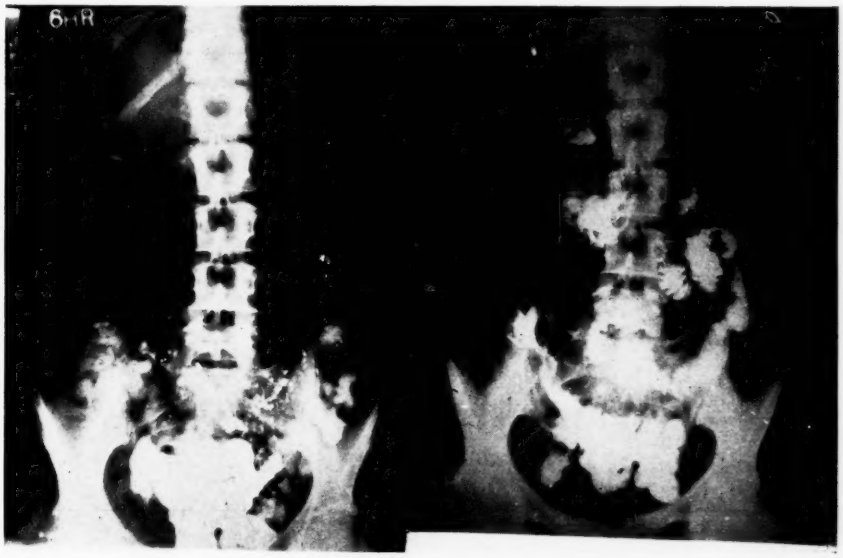

Fig. 2.—Patient E. J. The barium and water series is shown at the left and the allergic meal at the right. The comparative variations seen here are those of change in motility, dilatation of jejunal loops, and segmentation, apparently due to spasm.

E. J., Family history of allergy; urticaria twice since infancy; constipation, abdominal pain, and flatus after whole milk but able to take skimmed milk.

M. M., Asthma for two years; less frequent on milk- and chocolate-free diet; dust reaction, ++; other inhalants and foods, negative; trials with milk two times negative, two times positive; when chocolate added to milk had urticaria in two hours, asthma in four hours, lasting for 20 hours.

C. Z., Asthma for two years; pollen and dust injections improved him slightly; marked improvement on milk-free diet; severe asthma on addition of milk and during milk series.

TABLE II

GUINEA PIG STUDY

PIG NO.	SERIES 1 SMALL INTESTINE PATTERN	SERIES 2 SMALL INTESTINE PATTERN	SERIES 3 SMALL INTESTINE PATTERN	SERIES 4 SMALL INTESTINE P.N.* PATTERN	REMARKS
1	Normal	None	Normal†		Series 2, aspiration meal; Dale positive
2	Normal	Normal	Normal†		I.V. egg white, 50,000 units; anaphylaxis fatal in four minutes
3	Normal	None	Normal†		Series 2, insufficient barium; I.V. egg white, 50,000 units; anaphylaxis fatal in twenty minutes
4	Normal	Normal	Normal†		
5	Normal	Normal	Normal†		I.V. egg white, 50,000 units; anaphylaxis with recovery; Dale positive
6	Normal	Normal	50 minutes; normal	100,000; normal	Series 4, mild anaphylaxis
7	Normal	Normal	50 minutes; unsatisfactory	100,000; normal	Series 4, mild anaphylaxis
8	Normal	Normal	60 minutes; normal	100,000; normal	Series 4, mild anaphylaxis; Dale positive
9	Normal	Normal	260 minutes; normal	100,000; normal	Series 2, insufficient barium; Series 3, 4, mild anaphylaxis; Dale positive
10	Normal	Normal	260 minutes; normal	100,000; normal	Series 3, mild anaphylaxis; Series 4, anaphylaxis fatal in five minutes
11		Normal		50,000; normal	Series 4, no anaphylaxis; Dale positive
12		Unsatisfactory			Series 2, insufficient barium
13		Unsatisfactory			Series 2, insufficient barium
14		Normal			
15		Normal			
16		Normal		50,000; normal	Series 4, moderate anaphylaxis; Dale positive
17		Normal		50 000; normal	Series 4, mild anaphylaxis
18		Normal			
19		Normal			
20		None			Insufficient barium

*P.N., protein nitrogen units injected.

†Sensitized pigs.

Series 1. Control. Barium and water meal.

Series 2. Control. Barium and egg white meal.

Series 3. Sensitized pigs. Barium, egg white, and water meal. Remainder of guinea pigs fed 10 c.c. of undiluted egg white followed by barium and water meal indicated number of minutes later.

Series 4. Sensitized pigs. Barium and water meal. Intravenous shocking dose of 1:10 egg white; total amount of 0.4 to 1.0 c.c. given when intestinal filling noted by fluoroscopy.

Remarks: When noted under series number, symptoms occurred at that time. Within one week of attempt to shock by oral or intravenous route, Dale tests were carried out in some animals where sensitivity had not been proved.

TABLE II—CONT'D

PIG NO.	SERIES 1 SMALL INTESTINE PATTERN	SERIES 2 SMALL INTESTINE PATTERN	SERIES 3 SMALL INTESTINE PATTERN	SERIES 4 SMALL INTESTINE P.N.* PATTERN	REMARKS
21		Normal	Normal†	10,000; normal	Series 3, no anaphylaxis; Series 4, no anaphylaxis; Dale positive
22		Normal		120,000; normal	Series 4, no anaphylaxis; Dale positive
23		Normal		50,000; normal	Series 4, mild anaphylaxis
24		Normal	Normal†		Series 3, minimal anaphylaxis
25		Normal (?)		140,000; normal	Series 4, severe anaphylaxis; Dale positive
26		Normal			
27		Normal		140,000; normal	Series 4, mild anaphylaxis; Dale positive
28		Normal		150,000; normal	Series 4, mild anaphylaxis; Dale positive
29		Normal		140,000; normal	Series 4, anaphylaxis fatal in seven minutes
30		Normal			

EXPERIMENTAL STUDIES

It was felt that by simplifying the conditions of the experiment and working with serial x-rays of guinea pigs fed a specific food after parenteral sensitization to it, further insight might be obtained as to the mechanism of alteration of the pattern, whether it was due to edema of the mucous membrane or of the submucosa or to spasm of the intestinal wall.

Variation in the gastric emptying time even with a plain barium and water meal was found to be so great as to render it valueless for comparison with egg white meals. Technical difficulty in carrying out a barium enema made the study of the colon unsatisfactory.

The guinea pigs used in the investigation of the small bowel pattern weighed from 500 to 700 Gm. Prior to each x-ray series they were starved for forty-eight hours to improve the visualization which might be obscured by intestinal contents.

A mixture of pure barium sulfate and water was slowly introduced into the oral pharynx and the animal allowed to ingest the material in the normal manner. Progress of the meal was observed fluoroscopically and serial roentgenograms were taken at the time of optimal filling. The radiographic technique was 200 Ma., 40-45 kilovolt peak, 17-inch distance, $\frac{1}{120}$ second, using intensifying screens.

The procedure was repeated with a mixture of barium, egg white, and water, and no change due to food was apparent in the pattern. The same animals were then injected with 50,000 protein nitrogen units of

1:10 dilution of egg white and re-examined after three weeks. Various methods were used for the barium study of the sensitized pigs: (1) a barium, egg white, and water mixture similar to the previously employed mixture was given; (2) 10 c.c. of undiluted egg white were administered orally, followed by the normal barium and water mixture, examination was then made at hourly intervals up to five hours; (3) shock doses of 1:10 egg white were given intravenously after the small bowel had been outlined by a barium meal.

Two of the sensitized pigs with orally administered egg white exhibited mild anaphylactic symptoms but recovered. The remainder showed no symptoms. Several of the group given intravenous egg white recovered after severe anaphylaxis and several died. The pigs given oral egg white which did not show symptoms of anaphylaxis were sacrificed and positive Dale reactions obtained. The tabulated results of this study will be found in Table II.

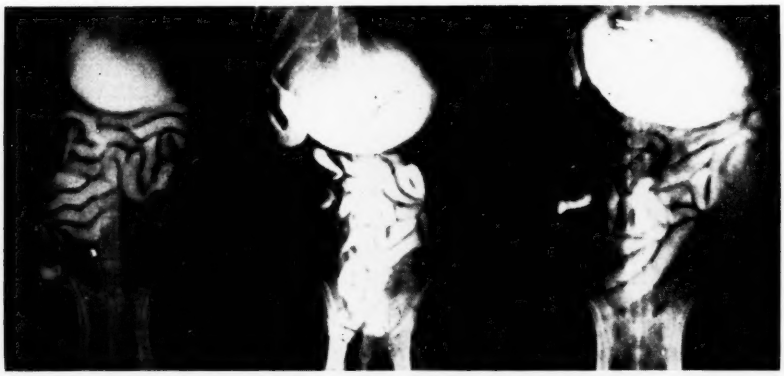

Fig. 3.—Guinea pig study. A control series of barium and water is shown at the left to illustrate the normal pattern of the small intestine. The film in the middle is the nonsensitized pig with a barium and egg white meal. The film at the right was taken at the height of severe anaphylactic shock following intravenous injection of the allergen. No variation among these three films is apparent.

Quite contrary to our expectations, no alteration in the small bowel pattern was noted in the sensitized pigs, even when the pigs were in fatal anaphylactic shock (Fig. 3).

COMMENT

This study indicates that in the allergic patient examination of the pattern of the small intestine may prove of some diagnostic aid in cases where the findings show marked deviation from the normal. In those cases showing minimal to moderate changes, extreme caution must be employed in interpreting the results, because Pendergrass[10] and coworkers showed minimal to moderate variation in the small bowel patterns of normal individuals when 50 per cent glucose, egg white, or olive oil was introduced with the barium. Furthermore, Macy[14] and

his co-workers, in a well-controlled study with barium and water and barium and milk meals, showed variation in the intestinal motility of the milk meal as compared with the control.

SUMMARY

1. The authors feel that in man the response of the small intestine to an allergen can be demonstrated roentgenographically in some patients with gastrointestinal allergy.

2. As an aid to diagnosis, however, it is of limited usefulness, because of the influence of nutritive foods on the behavior of the small intestine.

3. In sensitized guinea pigs no visual alteration in the small bowel pattern occurred during anaphylaxis.

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DISCUSSION

DR. IRVING GRAY.—The diagnosis of food allergy presents many difficulties with which an audience such as this is thoroughly familiar. Any method which will aid in more clearly defining or evaluating the question of the specific food allergy is welcome to the armamentarium of the internist as well as the specialist. Dr. Wing and his co-workers are to be complimented on their most stimulating presentation. The authors have called attention to the alteration in the x-ray pattern of the small intestine in three of nine patients suspected of having food allergy. Although this series may be small, the fact that in one-third of the cases there is evidence of pronounced deviation from the normal on x-ray study of the small intestine under the experimental conditions as described merits further intensive studies in the manner suggested by the authors. The alteration in the x-ray pattern is similar to that described in the deficiency state. However, it should not be difficult to exclude a deficiency state or other disease, and with the technique used as described by Dr. Wing, a diagnosis of food allergy may be strongly suspected if there are distinct deviations in the pattern of the small bowel. Inasmuch as skin testing with food allergens presents definite limitations, the x-ray study of the small bowel may prove a valuable aid in arriving at a diagnosis of food allergy.

It is well known that in the sensitized guinea pig there is a positive reaction of the smooth muscle of the small intestine on testing by Dale method. I can well appreciate Dr. Wing's surprise that he found no alteration in the x-ray pattern in the small intestine in his experimental studies on the sensitized guinea pig. The question might reasonably be asked, Is the spasm of smooth muscle the responsible factor for the alteration in the x-ray pattern? In our studies on the rhesus monkey after passive sensitization of the mucous membrane of the gastrointestinal tract, we found the primary lesion to be a marked edema of the mucous membrane as proved by histologic examination. In studies on the monkey we found occasional spasm of the small intestine. It was our opinion that the spasm was secondary to the tremendous edema of the mucous membrane at the site of the allergic reaction and that the smooth muscle was not the seat of the reaction. Dr. Murray Albert studied the question as to whether or not the smooth muscle in the monkey was sensitized after intravenous administration with sera of high reaginic titer. He found, by Dale technique, that there was no sensitization of smooth muscle. In a subsequent study, Dr. Albert sensitized the monkey with precipitins intravenously, and study of the smooth muscle by Dale technique failed to show any evidence of contraction or spasm following antigenic stimulation. From these observations we are inclined to believe that the massive edema of mucous membrane in the gastrointestinal tract that occurs in the experimental conditions may be present in individuals with food allergy and that this edema may be responsible for the various alterations found in the x-ray pattern.

In our studies of the human ileum and colon after passive sensitization we likewise noted the pronounced inflammatory reaction at the site of sensitization. With the normal bowel in situ acting as the seat of such a reaction, pronounced variations in the x-ray pattern, as described by Dr. Wing, are readily understandable.

In his x-ray studies of children with food allergy, Dr. Fries noted delay in the gastric emptying time. He raised the question as to whether or not there might possibly be some degree of pylorospasm. It appears to me that the pronounced edema of the mucous membrane in the region of the pylorus may well account for the delay in evacuation of the barium-filled stomach.

This stimulating presentation by Dr. Wing should encourage further studies with the technique that he has outlined in the hope that from more extensive observations and reports the method may prove of value.

Dr. Joseph H. Fries.—I am now in the process of analyzing some thirty allergic children with gastrointestinal disturbances following the ingestion of specific foods. We also have found definite disturbances in the small intestine in only a small percentage of the cases.

Now, if one conceives the small intestine as a relatively simple tube, one might expect few visual changes. True enough, there are changes in the caliber and motility of the organ and in segmentation.

On the other hand, the stomach is a far more picturesque organ, it is more differentiated, and most of our positive findings were observed in this particular region.

SKIN REACTIONS TO ELECTROPHORETIC FRACTIONS OF TIMOTHY POLLEN EXTRACT

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IT HAS been shown in previous communications[1] that the electrophoretic technique of Tiselius[2] may be used to fractionate aqueous extracts of giant and dwarf ragweed pollens. By this method it has been shown that each of these pollen extracts contains a major, colorless, negatively charged, slow-moving component and usually six or more minor pigmented components. In the case of giant ragweed pollen it was possible to separate the fastest moving pigment in the ascending arm of the electrophoresis cell. In this way the skin reactivity of the fast-moving pigment could be compared on a nitrogen basis with the skin reactivity of the slow-moving, major, colorless component, or USG fraction. It was found that the fast pigment was almost, if not equally, skin reactive to the USG fraction by direct tests on hay fever cases. Both of these fractions also showed the phenomenon of passive transfer. It was deduced that the pigments with intermediate velocities would also show activity and that the colorless constituent might conceivably be bound to pigment groups to provide for the increased electric mobility observed. This latter hypothesis, however, has not as yet been tested by exhaustion techniques.

We have now electrophoretically fractionated timothy pollen extract. As in the case of ragweed pollen extract, there is present a major, colorless, slow-moving component (in timothy) which is apparently electrophoretically homogeneous (UST–unpigmented, slow, timothy). In addition, there are also present about six or more pigmented components. However, the fastest moving pigment cannot be readily isolated by itself. We have, therefore, tested the skin reactivities of mixtures of the fast-moving pigments and the pigments moving with intermediate velocities. As with the ragweed extracts, it has been found that both the colorless and pigmented components are biologically active.

METHOD

The electrophoretic technique of Tiselius[2] has been employed. The curves have been analyzed by means of the conventional Toepler-

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Tiselius method as well as by the Longsworth[3] and Philpot-Svensson[4] devices. All of these methods showed that in aqueous timothy pollen extracts between pH 6 and 8 there was present a major fraction, colorless, slow-moving, probably slightly negatively charged, as well as the negative pigment fractions to be discussed in detail.

Timothy pollen obtained from commercial sources was extracted in a Soxhlet filter and kept in the icebox until used. About 5 gm. of pollen were dissolved in 50 c.c. of 0.02M phosphate buffer at pH 7.6 containing 0.15 sodium chloride. Occasionally more dilute solutions were used. In several instances during separation experiments, sodium acetate itself was used instead of a buffer. In this way the conductance of the solution was somewhat less, and the separation of the colorless fraction was facilitated.

The extracts, either with or without 1:5,000 merthiolate, were examined in the electrophoresis cells. About 1:5,000 merthiolate was added to solutions of UST containing from 0.2 to 0.6 mg. total nitrogen per cubic centimeter if no merthiolate had been added previously. After testing for steriliy, dilutions of 0.1 mg. of nitrogen per cubic centimeter and higher were made in buffered saline. Tests were done in at least duplicate with each dilution and occasionally in triplicate. Both the upper arm and back were employed with no difference in the results, although the degree of reaction occasionally varied with the site. After first testing with a dilute solution, progressions were made up or down as the degree of skin reactivity indicated.

To obtain a good sample of the UST fraction usually required an electrophoretic run of between twenty and forty-eight hours at 2° C., depending on the field strength. Since this was obtained at the descending boundary by using a compensation technique, the colorless fraction (UST) remained behind with the pigments migrating out of this fraction. This will be described in detail subsequently. At the ascending boundary it was relatively simple to withdraw by means of a hypodermic syringe and slow suction different pigment samples as they appeared in the buffer above the original pigmented timothy extract. In this way two different samples of pigments were obtained, first, the fast pigment mixtures (FPT), and second, pigment mixtures with velocities intermediate between the UST fractions and the FPT fractions (MPT). Our experimental data, therefore, deal with three different fractions, repeated here for clarity:

1. UST—Unpigmented, slow timothy (electrophoretically homogeneous).

2. MPT—Pigmented mixtures with intermediary velocities (UST-free).

3. FPT—Pigmented mixtures with the most rapid velocities (UST-free).

THE ELECTROPHORETIC PATTERN OF TIMOTHY GRASS

In Fig. I is an electrophoretic run of timothy grass pollen extract at about pH 7.4 for twenty-two hours. The time is given in the white circles under the ascending boundary for each pair (ascending and descending) of boundaries. The arrow pointing to the left in the first half-hour photograph illustrates the descending boundary whereas the arrow pointing to the right illustrates the ascending boundary. The ascending boundary shows the UST fraction remaining behind, but because the pigments are migrating faster into the buffer they are present in dilute solution and more light goes through. On the descending side, less light goes through and the photograph is shaded. This is because most of the pigment is present and mixed with the UST fraction.

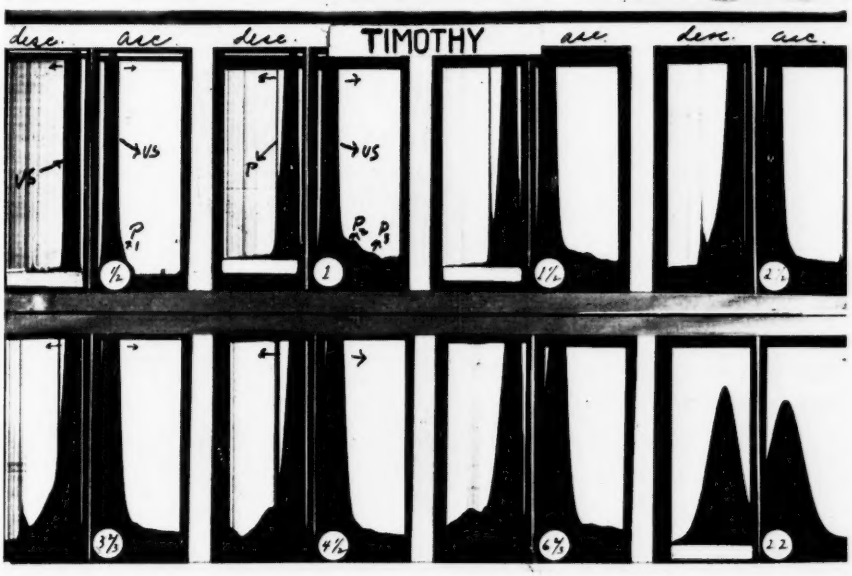

Fig. 1.—Electrophoretic analysis of timothy pollen extract from one-half to twenty-two hours. Base lines have been *drawn* in on the descending side for one-half, one, one and one-half, and twenty-two hours. *US* indicates the colorless fraction; *P*, a pigment fraction.

The ascending and descending boundaries are not quite symmetrical. Whereas in the half-hour photograph there is a fast pigment moving out in both, there are indications that two other (P_2, P_3) pigments are present. However, three pigments are obvious on the ascending boundary after one hour. These are essentially the pigment mixtures which we have designated as FPT. After one hour at least three pigments are still visible, but one of the pigments has migrated out of the picture and the third pigment forming is new. Inspection of the ascending boundaries at one and one-half, two and one-half, three and one-half, four and one-half, and six and two-thirds hours reveals the pigments migrating out of the solution into the ascending boundary.

It is these pigments which have been isolated to form essentially the MPT fraction. Comparison again of the ascending boundary and descending boundary in each case shows the shaded portion on the left to be different from the right except at twenty-two hours. This asymmetry is not due to a boundary anomaly but is probably due to complex formation of the pigments with one another. The faster pigments migrate through the slower moving pigments and leave the colorless fraction UST behind. To those unaccustomed to interpreting this type of pattern, it might appear that the colorless fraction is not evident. By inspecting the twenty-two-hour pattern, the colorless fraction, the UST fraction, which is readily isolated at the descending boundary by the compensation technique, is observed. The symmetry and smoothness of the curve indicate that this is an electrophoretically homogeneous component of timothy and corresponds in all likelihood to a single substance or a mixture of very closely related substances. The position of this fraction in the one-half hour pattern is indicated by the arrows labeled US. The US fractions occupy the same positions in all of the patterns, remaining, for practical purposes, stationary. It is occasionally difficult to get a water-white solution of timothy but by running the fractionations for a suitable length of time, the last trace of pigment separates, and a perfectly water-white solution is left behind. We have obtained UST fractions containing nitrogen as high as 0.6 mg. per cubic centimeter. It is of interest that the fast-moving pigment containing 0.006 mg. of nitrogen per cubic centimeter was about equal in color intensity to a whole timothy extract containing 0.1 mg. of nitrogen per cubic centimeter. In other words, this is a very simple way of concentrating the pigment, as may be expected.

The black areas under the curves with base lines are approximately proportional to the concentration of each of the constituents. Base lines are indicated only on the descending boundary of the first three patterns and of the last (twenty-two hours). Similar base lines could be drawn for the other boundaries. Evidently the US fraction is the major fraction on the basis of this method of measurement.

DIRECT TESTS OF SKIN REACTIVITY

A group of normal controls showed no skin reaction to any of the fractions containing 0. 1 mg. of nitrogen per cubic centimeter. Seventeen cases of constitutional hypersensitiveness were skin tested by the technique described in the section on Methods. In Table I is a group of nine patients with constitutional hypersensitiveness who were being treated for clinical symptoms of ragweed hay fever but who had no symptoms of grass hay fever. Of this group, five showed skin reactions to the two timothy fractions tested. In Case 2, the UST fraction showed

greater skin reactivity than the pigment fraction. In Cases 3, 6, and 7 the skin reactions were about the same to both fractions. The pigmented fraction showed greater reactivity in Case 4. In general, therefore, by

TABLE I

UNTREATED CASES

TOTAL N	1	2	3	4	5	6	7	8	9
UST 0.001	0 0	0 0	0 0	0 0	– –	– –	– –	0 0	2+ 2+
MPT 0.001	0 0	0 0	0 0	0 0	– –	– –	– –	0 0	2+ 2+
UST 0.01	0 0	– –	0 0	0 0	0 0	+ +	0 0	0 0	– –
MPT 0.01	0 0	– –	0 0	0 0	0 0	+ +	0 0	0 0	– –
UST 0.1	– –	4+ *4+	± ±	– –	+ +	2+ 2+	0 0	0 0	– –
MPT 0.1	– –	2+ *2+	± ±	– –	2+ 2+	2+ 2+	0 0	0 0	– –
Remarks						Hypersensitive skin	Diabetes mellitus		0.0001 UST + 0.0001 FPT +

–, Not tested.
All controls negative.
FPT used in first four cases negative.
*Repeated one week later.

TABLE II

TREATED CASES

TOTAL N	1	2	3	4	5	6	7	8
UST 0.0001	– –	0 0	0 0	0 0	†0 †0	– –	0 0	– –
MPT 0.0001	– –	0 0	0 0	0 0	†0 †0	– –	0 0	– –
FPT 0.0001	– –	0 0	0 0	0 0	†0 †0	– –	0 0	– –
UST 0.001	0 0	+ +	+ +	– –	± ±	– –	– –	– –
MPT 0.001	0 0	2+ 2+	2+ 2+	– –	+ +	– –	– –	– –
FPT 0.001	0 0	+ +	+ +	– –	+ +	– –	– –	– –
UST 0.01	2+ 2+	2– 2–	3+ *+ 3+	+ +	2+ †+ 2+	3+ ‡2+	2+ §3+	+ 0¶ +
MPT 0.01	0 0	4– 4–	3+ *+ 3+	2+ 2+	2+ †+ 2+	+ ‡+	+ §2+	+ +
FPT 0.01	0 0	4– 4–	2+ *+ 2+	2+ 2+	2+ †+ 2+	+ –	+ 2+‖ –	– –
UST 0.1	2+ 2+	– –	– –	– –	– –	– –	– –	0 ±¶ 0
MPT 0.1	+ +	– –	– –	– –	– –	– –	– –	2+ +¶ 2+
Remarks	All tests on back 0.1 on arm gave US2∓ MP∓	Back used	Patient tested 3 separate occasions	Hypersensitive skin. Arm used	Done on arm. Quantities different	Arm used	Arm used	Patient not treated since 1/41

–, Not tested.
All controls negative.
FPT 0.1 not used.
*Done on arm.
†Done on back.
‡Done one week later.
§Repeat one week later.
‖Variance in result.
¶Var. in result on repeat.

direct skin testing the pigments with intermediate electrophoretic velocities have about the same skin reactivity as the colorless fraction, although one fraction may be slightly more active than the other. This

is made all the more evident by examining the results at higher dilution. If the solutions were diluted 1:10, the reaction essentially disappeared. The difference in skin reactivity of the pigmented fraction and the colorless fraction is therefore located, in all likelihood, within an order of magnitude.

Of a group of patients with clinical symptoms of grass hay fever and who were positive on skin testing, data on eight patients are presented in Table II. These treated patients with clinical symptoms show a greater degree of skin hypersensitiveness than the untreated patients with minor grass symptoms. In addition, this group of patients was also tested to the fastest pigments as well as to the pigments with the intermediate electrical mobility. All of these patients showed skin reactions to solutions containing 0.01 mg. of nitrogen per cubic centimeter. Case 1 showed in the dilution of 0.01 mg. of nitrogen per cubic centimeter as well as in higher dilution that the UST fraction showed greater skin reactions than the pigmented fraction. In Case 2, on the other hand, the intermediate pigment had the greatest degree of skin reactivity. This was evident by examination of the data in the dilution of 0.01 mg. of nitrogen per cubic centimeter and the higher dilution. It was thought at first that a similar phenomenon was present in Case 3 but the differences are not considered significant. In Case 4 the pigments seem to be slightly more skin reactive than the colorless fraction. In Case 5 the skin reactivities are essentially the same. In Case 6, as well as in Case 7, the colorless fraction is more skin reactive. The data in Case 8 are inconclusive. These data for treated cases bear out the facts found for untreated cases as well as previous data on the ragweed fractions electrophoretically prepared.

PASSIVE TRANSFER

Table III illustrates that the UST and FPT fractions show the phenomenon of passive transfer with the serum of patient Che, and of patient Stu to a lesser extent. Serum Che was from a treated case of hay fever. Patient Gre was a ragweed hay fever case but had not been treated for timothy symptoms. Patient Stu had had incomplete timothy treatment. Sites 3 and 4, sensitized with an allergic serum which did not react, are good controls for those sites which did react. The extent of the reaction of the UST and FPT fractions in this experiment is about the same, both showing unequivocally the phenomenon of passive transfer.

Table IV is an illustration of a preliminary experiment to ascertain if there is any difference in the ability of the different fractions to exhaust sensitized sites. More extensive experiments are planned in collaboration with Dr. Gregory Shwartzman if circumstances permit.

The serum of Che was mixed with different quantities of UST and MPT fractions. After one hour rather large reactions were present at those sites which had received even dilute solutions of the antigen. Three days later, all of the sites, including the control, Gre, which was studied in Table III, were injected with 0.025 c.c. of the UST fraction containing 0.01 mg. of nitrogen per cubic centimeter. All of the sites still reacted except the control sites. However, the sites which had originally been treated with the UST fraction seemed to have been exhausted more by initial reaction with the UST fraction than with the MPT fraction. Thus, on the second injection, sites 1 and 2 show plus and plus-minus reactions, whereas sites 3 and 4 as well as site 5, a control,

TABLE III

PASSIVE TRANSFER—SUBJECT ENG

SITES	4/1/42 INJECTION*	4/3/42 INJECTION†	15 MIN.
1	Che + B. S.	UST 0.01	2+
2	Che + B. S.	FPT 0.01	2+
3	Gre + B. S.	UST 0.01	0
4	Gre + B. S.	FPT 0.01	0
5	Stu + B. S.	UST 0.01	+
6	Stu + B. S.	FPT 0.01	+
7	B. S.	UST 0.01	0
8	B. S.	FPT 0.01	0

*Total volume injection in each site, 0.1 c.c.
†0.025 c.c. injected.

TABLE IV

PASSIVE TRANSFER—SUBJECT DEM

SITES	3/27/42 INJECTION*	1 HOUR	3/30/42 INJECTION†	10 MIN.
1	UST 0.001 + Che	3+	UST 0.01	+
2	UST 0.01 + Che	3+	UST 0.01	±
3	MPT 0.001 + Che	3+	UST 0.01	2+
4	MPT 0.01 + Che	3+	UST 0.01	2+
5	B. Saline + Che	0	UST 0.01	2+
6	B. Saline	0	UST 0.01	0
7	B. Saline + Gre	0	UST 0.01	0

*Total volume injection in each site, 0.1 c.c.
†0.025 c.c. injected.

showed two plus reactions. It is realized that these experiments are essentially preliminary and merely confirm the fact that our fractions show the phenomenon of passive transfer rather than that any one fraction is different from the other. Experiments similar to those illustrated in Table III have been done on other subjects with the same results, the phenomenon of passive transfer being shown repeatedly by all of our fractions. Certain of these experiments were suggestive, as the data in Table IV imply, that the pigmented fractions did not exhaust the sites as readily as the US fractions. However, differences in diffusibility or tissue reaction velocity could conceivably account for

these differences and we do not believe that we have shown any definite evidence of differences in immunologic specificity. This is discussed in more detail in the next section.

DISCUSSION

Preliminary data obtained by means of the ultracentrifuge and diffusion methods indicate that all of the constituents we have just been discussing are of small molecular size. Prolonged ultracentrifugation of timothy extract at 150,000g did not result in any definite schlieren band forming after two hours at room temperature. However, as the temperature rose gradually to about 37° C., a band rather suddenly formed, indicating that in all likelihood at this temperature denaturation had occurred.

As shown in a previous report, the electrophoretically homogeneous, colorless fraction of giant ragweed (trifidin) and the similar fraction of dwarf ragweed (artefolin) have molecular weights close to 5,000. We have not as yet been able to determine the value for the molecular weight of the colorless fraction in timothy because of the small size, but it is apparently not more than 5,000 and probably less.

The question which arises and which will have to be solved by immunologic experiments is whether we are dealing with a colorless antigen common to the US fraction and all of the different pigments or whether we have antigens which are not so closely related. Whether or not an individual would become sensitized to the very fast pigment as easily as to the colorless fraction would depend, of course, upon all of those factors which have to do with the natural development of antibodies, including (1) the rate at which the components pass into the body; (2) the amount of these components; and (3) the ability that these components have to form antibodies.

In view of the low molecular weight of these substances, it seems highly likely that unless special solubility relations are present in the mucosa of the nose and of the lungs, they would pass by diffusion with relatively equal velocities. More of the colorless material, however, is present than any of the pigment. Indeed, more of the colorless material is present in all likelihood than all of the pigmented molecules together.

In one experiment the electric mobilities of three of the pigments in the ascending side were determined. These mobilities were 0.94 μ per second, 2.06 μ per second, and 3.3 μ per second. The very high electric mobility of 3.3 μ per second is more than thirty times the electric mobility of the unpigmented fraction. In other words, the net electrical charge of the fast-moving pigment is very different indeed from the colorless fraction. This indicates that there are present groups which are highly ionized at the pH's studied. However, the presence of these pigment groups need not alter the immunologic specificity to any great extent. If the pigment groups are all attached to a common antigen

similar to the US fraction, the pigment could decrease the immunologic activity of the common or "master" antigen and, in addition, confer new immunologic specificity. Which one of these occurs cannot be discovered from this report. It is hoped that future experimentation with electrophoretically isolated fractions will clarify this important and difficult aspect of the nature of the various antigens present in timothy pollen extract.

SUMMARY

1. Timothy pollen extract has been fractionated electrophoretically.

2. As in the case of ragweed pollen extract, there is present a major, colorless, slow-moving component which is electrophoretically homogeneous at pH 7.4.

3. In addition, there are also present six or more negatively charged, pigmented components which migrate more rapidly than the colorless fraction.

4. On direct skin tests, the slow-moving, colorless component, the fast pigments, and the intermediate pigments showed about the same skin reactivity.

5. On passive transfer, both the pigments and the colorless fraction also showed positive reactions.

6. Preliminary data obtained by means of the ultracentrifuge and diffusion techniques indicate that all of the constituents under discussion are of small molecular size compared with ordinary proteins.

7. The diffusion rate of the colorless fraction of timothy is very close to the analogous component of ragweed.

8. Questions arising connected with the immunologic specificity of the various fractions are briefly discussed.

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DISCUSSION

DR. ALEXANDER.—There are features of this presentation that seem to have importance. One is that we are dealing here with new material. Dr. Abramson has utilized methods employed by protein chemists, the ultracentrifuge, and Tiselius apparatus and thereby has secured some new material. Although it may be debatable as to whether these are proteins with a molecular weight of 5,000 or less, nevertheless they are something new in ragweed extract which we did not know existed.

It seems that by utilizing such material there may be an entirely new approach to this subject. For years investigators have tried to isolate the active fraction of pollen extract. They have done this by chemical methods, particularly by precipitation, and no one has succeeded as yet in finding a single active principle in any fraction of pollen extract as contrasted to the residue which is inactive.

Dr. Abramson's experiments suggest that his colorless fraction, his highly pigmented fraction, and the mixture all are active, and, not only that, it appears from the tables that they are all perhaps active to the same degree. That brings up the suggestion that perhaps we should stop seeking a single active component of pollen but rather consider what it is that may give activity to several fractions.

Now, in terms of chemistry, whether it may be a radical that is attached to these larger molecules or whether it be a physicochemical difference in aggregates of molecules that are common to all active fractions, it does seem that the approach to the problem now may be to break down analytically these really small molecules and perhaps find something common to each of them. This may act as haptene and thus is not some complex structure but perhaps something very simple.

Dr. Abramson's attitude toward this work seems very good. He has not tried to give final answer to anything, but merely announced a point of departure. I think it all looks very hopeful. We have something new from which to take off.

Dr. Vaughan.—I would like to be sure I have certain things right about this. Did Dr. Abramson state that these pigments are protein in nature? Are the more rapidly moving pigments necessarily of smaller molecular weight? Is there an arrangement in the series of apparatus so that you can detect a completely unpigmented substance? In other words, might there be something else moving along with the pigment?

Dr. Abramson.—I wish to express my indebtedness to Dr. Alexander for his careful discussion of the paper and to Dr. Vaughan for his pertinent questions.

Are the pigments protein in reaction? I should like to answer that question by discussing the reactions of both the colorless and the pigmented fractions. In our early experiments we did not get as dramatic protein reactions as those observed by Drs. Cooke, Stull, and their co-workers for their colorless fraction. However, in our last paper we have pointed out that our first fraction did not contain very much nitrogen. As soon as we obtained fractions with enough nitrogen in the colorless fraction, reactions more characteristic of proteins were obtained. Thus, in a recent series of experiments on the colorless fraction of timothy, the Millon, xanthoproteic, picric acid, phosphotungstic acid, trichloracetic acid, heat and dilute acetic acid, and sulfosalicylic acid tests were all positive. These tests are also positive for the colorless fractions in both ragweeds (artefolin and trifidin). The color of the pigments, themselves, interferes somewhat with the tests. However, positive tests were obtained with the pigments of timothy to Millon's reagent, phosphotungstic acid, trichloracetic acid, and sulfosalicylic acid. The pigment fractions are always more dilute than the US fractions. This is evident from the curve.

There is no reason why a protein molecule cannot have a molecular weight of 5,000, although it is true that it would be about the smallest protein molecule ever isolated. Nevertheless the boundary between a high molecular weight polypeptide and a low molecular weight protein is obscure, and, in all likelihood, a dividing line cannot be drawn with our present method of defining proteins.

Could a rapidly moving pigment group coupled with a small colorless radical account for the activity of the pigment? It is quite possible. Also, another colorless component could be present in the UST fraction, this second colorless component having the same electric mobility. The colorless fractions, UST as well as USG, could have colorless components within them of the same electric mobility which we could not find with our method. A colorless component of that type could be, for example, a polysaccharide with a net charge very close to zero. We should probably have to rely on chemical methods to determine a mixture of that sort, using, however, as our starting point, electrophoretically homogeneous fractions. Varying the pH or salt type might also lead to further fractionation. Indeed the method presents almost unlimited possibilities. Against the point of view, however, that the colorless component has two major fractions in it, is the following experiment. In the ultracentrifuge, the electrophoretically homogeneous US fraction of ragweed, at least, showed only one band. If there were both a small molecule and a large molecule with a mobility of the same order present, two bands should have been observed. However, we are keeping an open mind on that matter and will do our best to ascertain the exact nature of our colorless fraction.

Dr. Vaughan also asked if there is any relationship between the size of the molecule and the electric mobility. With molecules of the same size and shape in solutions of the same salt concentration and type, the electric mobility is proportional to the net charge. It is the potential, in general, at the surface which determines the speed of the ion.

In conclusion I might say that as we go over our curves and accumulate an increasing quantity of data, we see justification for the project not only because of the few questions regarding the nature of the pollen allergens which are answered but also because of the unsolved questions which each new set of records raises. As Dr. Alexander has pointed out, it is the solution of these questions which will probably lead to more definitive information on the nature of the pollen allergen.

RESPIRATORY ALLERGY FROM THE ARSPHENAMINES

Personal Case Report

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CUTANEOUS hypersensitivity to the arsphenamine drugs has been of fairly frequent occurrence and has been extensively described (Stokes,[1] Moore[2]). Respiratory hypersensitivity to these, however, judging from the scarcity of reports in the available literature, must be of great rarity. Klauder[3] reported the case of a physician who suffered both contact dermatitis and bronchial asthma from preparing neoarsphenamine for injection. Vaughan[4] mentioned a physician who developed asthma immediately on opening an ampule of neoarsphenamine.

Asthmatic attacks following the intravenous administration of neoarsphenamine in a patient who had never before had asthma have been described by de Beyrouth.[5] Stokes[1] states that arsphenamine asthma is a rare condition, usually associated with urticaria, coming on from twenty minutes to four or five hours after injection and promptly relieved by epinephrine. He states that "it argues a perhaps dangerous degree of sensitization."

In view of this apparent rarity of respiratory allergy from the arsphenamines, the following case is herewith presented.

CASE REPORT

As a practicing syphilologist, I have been continually exposed to antisyphilitic arsenicals for the past eight years. During the first five years of this period, no difficulties arose from exposure to or contact with these drugs. About three years ago, however, I began to develop respiratory symptoms in connection with the preparation of antisyphilitic arsenicals for injection. The symptoms were trifling at first but have gradually reached disagreeable proportions.

About one minute after opening an ampule of mapharsen, arsphenamine, or neoarsphenamine, an intense sneezing attack begins. Shortly after this, rhinorrhea sets in, ordinarily quite profuse. Along with this occurs the most distressing symptom, asthma. Respiration is difficult—noisy, musical râles being audible a few feet distant.

This symptomatic ensemble lasts about thirty minutes, as a rule, and then disappears. The intensity of the attack is directly proportional to the degree of exposure to the powdered or crystalline drug. Some attacks have followed the inhalation of air from the room in which the arsphenamine solutions were prepared. A subcutaneous injection of 0.5 c.c. of 1:1000 solution of epinephrine affords prompt, but not complete, relief. Wearing an ordinary surgical (cotton) mask when preparing the solutions did not appreciably lessen the ensuing symptoms. My present practice of having an assistant prepare the drug in a distant room, with wide-open window, affords me practically complete protection. Inhalation of tryparsamide and other crystalline and powdered drugs or chemicals has never caused me any respiratory difficulties.

Prior to my coming in contact with the arsphenamines, I had never had an attack of asthma, or hay fever, or any other allergic manifestation. However, subsequently, I have developed mild asthmatic attacks after severe exertion or after bouts of hearty laughter.

Inspection of my nose on March 1, 1942, showed a pale, edematous mucosa, suggestive of an allergic disturbance (Dr. C. C. Petheram). Microscopic examination of nasal smears showed, on one occasion, 54 per cent eosinophiles, and on another occasion, 100 per cent eosinophiles (Dr. Merle Moore).

Cutaneous Tests.—On Jan. 8, 1942, I applied patch tests of mapharsen and neoarsphenamine to my left forearm. Powder and solution (in the dilution used for intravenous injection) of both drugs were used, and the adhesive dressings were tightly sealed to prevent escape of content and subsequent inhalation. No symptoms occurred until about nineteen hours later when I was awakened by a severe attack of asthma. Inspection of the patches showed them to be airtight and in good position. The asthma had apparently been caused by absorption of the drug through the skin (transepidermal penetration). Removal of the patches was followed by relief in half an hour. Examination of the tested areas showed a mild reaction (erythema without vesication) to the mapharsen powder only. This erythema and attendant itching lasted for nearly three weeks. In view of the fact that the mapharsen solution produced no local reaction, that caused by the mapharsen powder probably represented primary irritant effect.

On March 16, 1942, I applied a drop of neoarsphenamine solution (6 per cent) to scratches on the forearm of my assistant and myself. About five minutes later my attention was directed to the site of application by a sense of irritation. Examination showed a giant urticarial plaque with pseudopods at the neoarsphenamine scratch. Control scratch tests were negative as was a scratch test on my assistant. Just about that time I had several explosive sneezes, followed by a suffusion of the conjunctivae and a sense of burning constriction in mid-chest. This was, in turn, followed by an intense attack of asthma which required epinephrine for relief. Care had been exercised that I did not inhale the drug; in fact the solution had been left over from a previous treatment which had caused me no respiratory difficulties. Because of the intensity of this attack, attempts at further experiments (ingestion, intravenous) were abandoned.

COMMENT

Klauder[3] attempted to explain the development of the respiratory (and cutaneous) allergy of his patient on previous intracutaneous testing with the drugs concerned. He recalled his experience that the majority of arsenical erythrodermas occurred in patients who had suffered paravenous administration of the drug. This theory fails to explain the development of my symptoms, for I have never previously been tested with arsphenamines or related compounds, nor have I ever had any injections of these, paravenous or otherwise. The explanation in my case is probably a simple one: repeated exposures by inhalation to the drugs finally resulted in sensitization.

The reliability of skin tests (patch, scratch) in determining cutaneous hypersensitivity to the arsphenamines has been subject to question. However, it would seem that the scratch test is an accurate indicator of respiratory sensitivity. Klauder's patient had strongly positive scratch tests to arsphenamine and neoarsphenamine but apparently an attack of

asthma was not precipitated by the scratch tests. In my own case, an intense attack of asthma followed absorption of the neoarsphenamine solution from the scarified area. One could scarcely wish a more convincing demonstration of sensitivity.

SUMMARY

1. A personal case of respiratory allergy from inhalation of the arsphenamines has been described.

2. Allergic testing of the suspected material was carried out as far as was deemed practical.

3. The reproduction of respiratory symptoms through transepidermal penetration instead of inhalation is described.

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MEDICAL ARTS BUILDING

HYPERSENSITIVITY TO SULFONAMIDES

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A NUMBER of cutaneous manifestations, including several cases of exfoliative dermatitis, after internal use of sulfonamides has been recorded. In these instances patch tests gave negative results. Occasional flare-up of eczematous lesions has also been observed after the ingestion of these drugs.

Several reports on the beneficial effect of sulfonamides externally in different skin diseases have been published and some cases of hypersensitivity have been observed. Probable increase in the number of such instances is to be expected as the use of sulfonamides in dermatology becomes more general.

Sams and Capland[1] described a case with a probable local sensitivity to sulfathiazole. Application of sulfathiazole powder in the external auditory canal was followed by the acute exacerbation of the dermatitis, and ingestion of 1 Gm. of the drug caused marked edema of the face and a generalized, patchy, erythematous dermatitis. Patch tests, however, gave negative results. Pillsbury[2] also observed a similar case.

In a previous report,[3] exacerbation of two cases of nummular dermatitis after the use of sulfathiazole in a glycerin paste was mentioned, but no true hypersensitivity was observed. During the past six months, this paste has been routinely used in the treatment of pyodermic skin diseases, and there have been five patients who reacted unfavorably to this type of therapy. Two of these displayed features of interest and will be described in detail.

CASE REPORTS

CASE 1.—An 18-year-old boy was first seen in October, 1941. His chief complaint was redness and pustular lesions on his chin in the beard area. He stated that in June he had had a small pustular lesion on the chin which had itched considerably; this was followed by a fine rash with slight swelling over the whole face. He applied some ointment and the eruption practically cleared. Pustular lesions recurred and hot boracic compresses and sulfathiazole, 30 per cent, in a glycerin paste were prescribed at the time of the first visit. His condition improved. He was again seen on January 21 with a recurrence of the eruption, but this time he presented a vesicular, weeping, and infected dermatitis which involved the forehead and beard area. Sulfathiazole in same strength and base was again prescribed and dermatitis improved, but in two days it relapsed and yellow impetiginized crusts were observed. He was admitted to the hospital on January 25 with erythema and swelling of the face, edema of the lower lids, and a follicular, papulovesicular eruption involving the whole body. Temperature was slightly elevated. Blood sulfathiazole level was 0.05 mg. per cent.

From the Section of Dermatology, Department of Medicine, Royal Victoria Hospital.

Past history revealed that he had had two attacks of poison ivy four and six years before.

Culture from a pustule on the face showed a heavy growth of staphylococci pyogenes and hemolytic streptococci. With soothing topical applications, the condition improved and he was discharged on February 24. Two days after discharge the patient was bothered with a severe nocturnal pruritus, and his skin lesions gradually became worse. He was readmitted to the hospital on March 4, with patches of a vesiculopapular eruption on the face with partially crusted lesions on the back, extremities, and face. The following patch tests were done:

1. Sulfathiazole, 5 and 30 per cent, in glycerin paste was strongly positive, but the control with the glycerin paste was negative.

2. Sulfathiazole, 10 per cent, in petrolatum base was negative.

3. Patch tests done with sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazine powder were positive.

4. Patch test was positive with sulfanilic acid powder, but in solution 1:1000 the reaction was doubtful. Considering the possible caustic effect of sulfanilic acid, no definite conclusions could be drawn. Sulfathiazole, 10 per cent, in paraffin and triethanolamine[4] base gave a positive reaction, but control with the base was also positive. There was no reaction to the adhesive and a test made with unguentum hydrargyri ammoniati in petrolatum base was negative. An intradermal test made with sodium sulfathiazole, 1:200, was positive, but the alkalinity of such a substance may cause a pseudoreaction.

On March 18, 2 Gm. of sulfathiazole were administered orally. Four hours after ingestion, the patient complained of intensive itching and burning over the body. Twelve hours later, he had developed a marked edema of the face, especially of lips and eyelids, and his temperature was 103° F. The old eczematous lesions on the face and hands became inflamed, and profuse weeping was noted. He felt nauseated and ill and some new lesions appeared on face. Blood sulfathiazole level after twenty-four hours was 2.5 mg. per cent.

The exacerbation faded after three days. Soothing topical treatment was given and the patient was discharged on March 30.

Case 2.—A. A., a 42-year-old barber, was first seen in May, 1941. He stated that he had been suffering for the last four years with a relapsing eruption on his face. External treatment, including x-ray therapy, had a temporary effect only. In the bearded area his face was covered with small superficial pustules, as were his forearms and the backs of his hands, typical of sycosis. A culture from a pustule on his face produced a heavy growth of staphylococci pyogenes.

Sulfathiazole, 30 per cent in a glycerin base, was applied to the lesions, and after four days of applications the face became slightly irritated. There was no improvement in the sycosis, and sulfanilamide in the same concentration and base was applied. This was followed by an immediate reaction with edema and a weeping dermatitis. Finally the sycosis healed after a series of staphylotoxoid and autogenous vaccine injections.

In September, 1941, a relapse occurred, with numerous pustules on chin and cheeks and similar lesions on the backs of the hands and mons veneris. The patient was admitted to the Royal Victoria Hospital.

Patch tests with sulfathiazole and sulfanilamide, 30 per cent in a glycerin paste, gave negative results on normal skin, but application of these pastes to the face was followed by localized redness and eczematous lesions. Sulfathiazole, 2 Gm. given internally, caused considerable edema of the face and hands which subsided within twenty-four hours, to be followed by a weeping dermatitis on the face and a fresh eruption of pustules on the hands and mons veneris. A culture from these pustules

again showed staphylococci pyogenes. However, there was no reaction on normal skin and no fever. After several weeks of soothing topical therapy and vaccine, the eruption healed.

DISCUSSION

In the first case, sensitivity to four sulfonamide compounds was demonstrated by patch tests. It is interesting to note that the vehicle seems to be of importance. Sulfathiazole in glycerin gave a markedly stronger reaction than pure sulfathiazole powder triturated with water, even if the former was used in a concentration of but 5 per cent. Petrolatum inhibited the effect of sulfathiazole, probably by mechanical insulation of the drug particles. This observation corroborates our clinical impression that sulfonamides in petrolatum are less effective.

The second case is more difficult to interpret. Here the sensitivity to external application was limited to the diseased area, and ingestion caused reactions in these same parts of the skin only. The other interesting feature was the complete failure to cure or improve the staphylococcic infection.

The history and course of both cases seem to suggest that the sensitivity was produced or at least increased by repeated external application of sulfonamides. Further studies will be necessary to prove this. Since the sulfonamides are indispensable in the treatment of various grave diseases, sensitivity to these drugs is a serious matter. Patch tests will be repeated at regular intervals in order to determine whether their sensitivity is permanent.

SUMMARY

Two cases suggesting hypersensitiveness to sulfonamide compounds are presented.

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CHRONIC OTORRHEA DUE TO FOOD SENSITIVITY

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A SURVEY of the literature substantiates the belief that chronically discharging ears in allergic subjects due to specific sensitivities are indeed rare.

Lewis,[1] in 1929, reported six cases of spontaneously perforating eardrums with serous discharge but, unfortunately, detailed and comprehensive analysis was not presented to prove a definite antigenic relationship in any of these cases.

Proetz,[2] in 1931, also mentions one case of a surgeon who was subject to spontaneous rupture of the eardrum with serous exudate; also Dean and associates[3] who mentioned, in 1937, a woman, 35 years of age, with chronic otorrhea for nine months. This patient also had a chronic vasomotor rhinitis, and, according to these authors, when the allergic rhinitis was brought under control, the ear stopped draining. Here again, in both cases described, no scientific proof of allergenic causes was established.

Two cases of chronic otorrhea which were definitely associated with specific food sensitivities were referred to me in the past two years and are herewith described.

CASE REPORTS

Case 1.—E. M., male, aged 15 years, was referred to me Feb. 10, 1940, because of persistent serous, and at times purulent, drainage from left ear. There was a history of asthmatic attacks and sneezing, rhinitis, and nasal congestion indicative of an allergy.

Family history revealed a mother with hay fever, sister with hay fever, and a grandmother with hay fever and asthma.

As an infant the patient suffered from eczema and he would vomit after being fed milk, and later eggs, although he could tolerate egg yolk without any intestinal symptoms.

When 11 months old, both eardrums were lanced because the child suffered from severe head colds accompanied by bronchial cough, running nose, sneezing, and a slight elevation in temperature. He was on ten ounces of milk, four times daily. His ears apparently healed thoroughly and stopped draining soon after his return from the hospital, although he appeared considerably emaciated and anemic.

When he was about 1 year old, meats (chicken and beef) were added to his diet. The father observed that invariably, following their ingestion, the boy would vomit and his temperature would subsequently rise to from 102 to 103° F., associated with a correspondingly elevated pulse rate. Coincidentally, some sneezing, sniffling, and congestion of the nasal mucous membranes ensued. Ingestion of these meats daily for a period of about one week would result in an asthmatic attack.

Because of the deleterious effect of the meats and eggs, the father cancelled these items from the diet when the child was 3 years of age, and this resulted in the elimination of the above-mentioned attacks.

When 5 years of age the child contracted measles, following which the ears started draining a profusely purulent material.

Since then, the father relates, about every three months the boy would undergo a siege of nausea and vomiting, severe headache over left eye, sneezing, running nose, and later asthma, which was followed by a persistently draining left ear, lasting approximately one month.

These attacks became more frequent and intense from year to year and especially after the readmission of meats and eggs into the diet in 1938.

Aside from history of food sensitivities, the boy also suffered from hay fever, associated with asthma in the fall of the year. Dusty environments produced violent attacks of sneezing and rhinitis. When fatigued, the catarrhal symptoms became aggravated.

During the course of an attack due to either foods or inhalants, his disposition changed to one of fretfulness and irritability.

Because of the insistence by the father in his belief that meat, especially beef, was one of the major causes of his draining ear together with the elevation in temperature and pulse, it was decided to remove all meats and other known food sensitivities from his diet until all symptoms subsided, and then to reintroduce a small portion of cooked beef. This experiment was carried out on 3 separate occasions with the resulting serous discharge from his ear associated with elevation of pulse and temperature each time.

In the course of these clinical experiments he was very thoroughly tested intradermally with the 200 foods and inhalant extracts. He reacted moderately to all of the meats (except pork), sweet corn, plum, walnut, cashew, potato, vanilla, egg, and chocolate, and slight to slight plus to strawberry, celery, asparagus, tomato, ginger, orange, lemon, and pineapple. The other food reactors were doubtful. He also gave a moderate reaction to dust and ragweed. Passive transfer tests were done and positive reactions to beef and eggs were prominent. He was instructed to avoid all meats, eggs, and the foods that were definitely suspected from the history.

Injections of ragweed and dust extracts were instituted. He continued to show gradual improvement with reference to his weight, disposition, general health, appetite, and ambition. His ear stopped draining, and he gained 8 pounds in one month.

Unfortunately, he moved to another town soon thereafter, but he complied with my written request to return for a personal check-up as to his progress on Feb. 28, 1942, approximately two years after I had first seen him.

The boy has not had a discharge from the ears in a period of two years, and he could eat with impunity meats and other foods to which he was once sensitive. He has grown two inches in height, gained 30 pounds, and seemed to be in perfect health.

CASE 2.—B. A., male, aged 8 years, was referred to me Nov. 19, 1941, because of a draining left ear and urticarial eruption one week previously.

The family history revealed one brother with seasonal hay fever and hives, an aunt with seasonal hay fever, and another aunt with asthma.

The past history as related by the mother revealed that feeding the infant Carnation milk or his formula mixture would evoke intense abdominal pains and cramps which caused him to double up and cry bitterly. These foods were consequently discontinued.

At the age of 2 years, following a siege of scarlet fever, the child developed a left mastoiditis with a discharging left ear. Mastoidectomy was performed at the age of 2 years and again at 4 years of age.

From April, 1937, the child's left ear kept draining almost continuously, mostly a serous discharge, which changed at times to a mucopurulent material.

These attacks of otorrhea would occur almost monthly and were always preceded by what the mother described as head colds, to which the child seemed unduly susceptible. These colds were accompanied by symptoms of otorrhea, nasal congestion, sneezing, coughing, and, at times, sore throat. He invariably complained of nausea with these colds, but the temperature remained normal with the exception of the times when the discharge from the ear became mucopurulent, at which times a slight elevation in temperature occurred.

Throughout these years, the boy was never completely well and exhibited the pale, anemic, washed-out appearance characteristic of a chronic ailment. His appetite was poor, and he was considerably underweight. He appeared thoroughly fatigued with a distinct lack of energy and vitality contrary to a normal boy of his age.

From her personal observation, the mother volunteered the information that after feeding the child Campbell's vegetable soup on two separate occasions symptoms of abdominal pain, nausea, and vomiting followed. She suspected tomatoes as perhaps being the culprit in producing these symptoms.

On Nov. 10, 1941, the day after he ate spaghetti with tomato sauce, he broke out for the first time with generalized hives. That night, his ear again started draining a serous material.

The child was thoroughly tested intradermally with about 200 foods and inhalants. Egg white produced a marked reaction on the first day of testing. He reacted slightly to chocolate, pork, and tomatoes, and to horse epithelium and horse serum. Dust produced a moderate reaction. The remainder of the tests were negative.

Passive transfer tests with egg and tomato were done, and a positive reaction to egg but not to the tomato was noted.

He was instructed to eliminate eggs entirely as well as the other foods to which he reacted. In the course of the next few days, the ear stopped discharging, he stopped complaining of gastrointestinal upsets, and his general health and appearance improved. He gained 3¾ pounds in the space of one week.

On Dec. 10, after being symptom-free for a period of approximately three weeks, his mother gave him a doughnut. The following two days the boy remained in bed vomiting and complaining of cramps. The third day after eating the doughnut, the left ear started to discharge a serous fluid and the urticarial eruption recurred.

After the ear stopped draining and the gastrointestinal symptoms completely subsided for a period of one month, he was instructed to drink one glass of tomato juice in order to ascertain more precisely whether tomatoes alone contributed to any of the boy's symptoms instead of eggs. The next day the mother reported that he broke out in generalized hives one-half hour after drinking the tomato juice, but the ear remained dry and he remained free of any gastrointestinal disturbances.

For the past nine months his ear has stopped draining completely, and he has had no further gastrointestinal upsets. He has gained steadily in weight with a distinct improvement in his color and general physical state. Above all, his interest in his schoolwork and surroundings has increased profoundly, keeping pace with a markedly improved disposition.

DISCUSSION

It is common knowledge that any tissue of the human body can enter into a specific antigen-antibody reaction, including the membranes of the middle and internal ear. As a so-called shock tissue, the tympanic membrane of the middle ear commonly enters into such allergic reactions because of its anatomical continuity with the membranes of the oral cavity and eustachian canal.

To allergists and otologists alike, the history of pain in the area around the ear, deafness, itching, and perhaps tinnitus in the sensitive individual in association with other allergic manifestations is both common and real. Objectively, such cases present a swollen, and sometimes reddened, tympanic membrane.

It is obvious, from the literature and from one's own practical experience, that the otologist encounters these patients before the allergist, and it is consequently evident that the otologists are more familiar with the picture of discharging ears or swollen eardrums due to sensitivity. As allergists, we are much too indifferent to the variegated complaints of pain, buzzing, and deafness of patients whose primarily more prominent symptoms of hay fever, allergic rhinitis, and asthma tend to subordinate the rest of the picture.

The spontaneous perforation of an eardrum in an allergic individual is encountered but rarely in the ordinary practice of allergy, although it is conceivable that such perforations can spontaneously occur. However, in the cases described, it becomes strikingly significant that the perforations of the drums followed childhood infections (measles in one case and scarlet fever in the other), and it is reasonable to assume theoretically that, because of the so-called lowered resistance of the membranes of the middle ear, they became natural sites for continuous insults, with subsequent edema and drainage. One must not lose sight of the fact that in both cases multiple manifestations of allergy were prominent, and their histories revealed that colds were consistent forerunners to the episodes of the discharging ears. That the discharge was but one phase of the generalized pathologic picture of nasal and sinus congestion is obvious.

It is, of course, necessary to distinguish between the prevalent cases of otorrhea due to an initial bacterial infection and those due to allergy. There is no question in my mind but that the two cases described belong in the latter category, since repeated clinical experimentations with the definite foods to which both individuals were sensitive resulted in the recurrence of the discharge after they had stopped draining.

The history of intercurrent purulent discharge at times is not to be interpreted as the basic cause of the discharge, since this phenomenon in effect parallels the common history of sinus and bronchial infections with purulent drainage secondarily superimposed on an already allergic membrane. The fact that no elevation in temperature was associated with the draining ear (as in the case of B. A.) unless a secondary infection set in would further enhance the diagnosis of an allergic otorrhea rather than a primary bacterial invader.

It is unfortunate that no smears of the drainage were made in either case for the presence or absence of eosinophiles. Although Hansel emphasizes the necessity of an increased eosinophile count to establish a basis of allergy, the absence of an increased count does not mitigate against such a diagnosis.

The differentiation between a swollen eardrum due to a primary bacterial infection and one due to a specific sensitivity assumes paramount importance, because paracentesis of a tympanic membrane due to specific antigens should be at all times condemned. It is obvious that, like the congestion of the mucous membranes of the nose or sinuses, this swelling will automatically diminish within twenty-four hours upon the avoidance of the specific antigenic causes or by the use of medication to shrink the membranes. The mechanical opening of an allergically swollen membrane always increases the hazard of a secondary infection.

Since this report was written, two cases of spontaneous perforation of the drum due to specific foods have been called to my attention. It becomes increasingly clear that considerably more attention must be paid to cases of chronic otorrhea which do not respond to proper otologic treatment since these cases will be found to be definitely allergic, especially if an antecedent history of allergy is obtained together with a coexisting history of other allergic disturbances in the individual.

Unnecessary surgical intervention can be obviated by (1) the correct identification of the cases as being allergic in character and (2) by the subsequent and detailed proper therapeutic approach.

SUMMARY

Two cases of apparent otorrhea due to food sensitivity are described.

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BIOTIN AND SKIN SENSITIVITY

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PHILADELPHIA, PA.

RECENT reports have appeared in the literature relative to hypersensitivity of given individuals to certain members of the vitamin B complex.[1] As far as is known, a skin contact reaction of the wheal and erythema type, following contact with biotin, has not as yet been recorded in the literature. Such a reaction was suspected in a laboratory worker and has subsequently been investigated.

This individual noticed red, itching patches on her arms and hands whenever mixing a dry dusty diet containing butter yellow and known members of the vitamin B complex. There were slightly raised areas in the center of the red patches which lasted about half an hour. Since this individual had been previously and repeatedly exposed to all the elements in the diet except biotin, this substance was suspected.

Accordingly, skin patch tests were employed, using the factors contacted by this individual when handling biotin. The substances tested were (1) rice and 3 per cent butter yellow in olive oil; (2) rice and the B complex including thiamine Cl, choline chloride, B_2, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, and biotin; (3) crystalline nicotinic acid; and (4) biotin concentrate.*

Since biotin gave a reaction within two hours, the above test substances were removed at the end of that time, the other substances thus serving as controls. The first test substance gave no skin reaction at all after two hours of application. The second, B complex, containing biotin, produced an area of erythema and central edema that lasted ten hours. The crystalline nicotinic acid produced an area of erythema without edema which subsided completely in an hour. The biotin concentrate, however, produced after the same period of contact a central, firm, raised, pale area with a surrounding zone of spreading erythema. This raised area was tense and shiny and persisted more than an hour after all redness in the other areas had vanished. At the end of thirty-six hours the central area was grayish tan and tender on palpation.

To rule out directly the other substances handled, as the offending agents, skin patch tests were done with thiamine chloride, riboflavin, pantothenic acid, vitamin B_6, 70 per cent alcohol, and 25 per cent hydrochloric acid (since most of the substances were tested as chlorides). All of these substances failed to produce a skin reaction after two hours of direct skin contact.

From The Lankenau Hospital Research Institute, Philadelphia.

*Concentrate No. 200, S. M. A. Corporation.

It should be noted that this individual did not get a reaction to biotin unless the skin had previously been cleansed with 70 per cent alcohol or unless nicotinic acid was present in the mixture; nor was itching noted at the site of reaction when biotin alone was applied to the cleansed skin surface. However, in conjunction with nicotinic acid, itching was present. It was further observed that biotin, in conjunction with nicotinic acid, applied to the uncleansed skin produced a reaction of the wheal and erythema type accompanied by itching. This, however, was not observed if the individual previously had applied a commercial cream to the exposed area. This latter fact was observed when measures were undertaken so that the individual could continue her work with biotin.

Skin tests were next performed with 25 μg of crystalline biotin dissolved in 1 c.c. of water. This solution was used as stock and half of it diluted serially by taking half the cubic centimeter of stock and adding 0.5 c.c. of distilled sterile water. The resulting solution was treated in a similar manner and so on until seven dilutions were obtained, including the stock solution. Three drops of each solution were applied to designated areas on the skin of the forearm previously cleansed with alcohol and allowed to remain in contact for one hour. At the end of this time, the first four dilutions produced a wheal and erythema, the erythema being least marked in the second instance. In dilutions 5 and 6 erythema alone was noted, and in the dilution 7 no reaction at all was observed.

SUMMARY

1. It has been shown that biotin can produce skin sensitivity in an individual as measured by the contact skin test.

2. Nicotinic acid apparently enhances the reaction on the uncleansed skin and exaggerates it (itching) on the cleansed and uncleansed skin surface.

3. It was further observed that the application of a commercial cream prevented a skin reaction from contact with biotin dust.

REFERENCE

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SEASONAL SOMNOLENCE, A POSSIBLE POLLEN ALLERGY

Case Report

Louis Sternberg, M.D.
New York, N. Y.

MIGRAINE epilepsy and Ménière's syndrome have been explained in certain instances upon an allergic basis. Other vague neurologic manifestations have also been reported in the literature by various authors. Pathologically these cases suggest an allergic reaction of the hyperemic or edematous type in the tissue of the nervous system.

Rowe[1] reported a case of epilepsy where pollen was thought to be the causative agent. Vaughan[2] described a patient who felt tired after the ingestion of any form of wheat and was full of energy when that food was eliminated. May[3] reported a similar case. Insomnia due to orange and blackberries has also been described by Rowe and Richet.[4] Crowe,[5] presented a case of cerebral edema which he was able to control by the elimination or the introduction of the offending food.

The following case is presented because the history, the clinical findings, and the course of events make it appear possible that it is an unusual sensitization reaction of the cerebral type to an air-borne allergen without any evidence of associated nasal or bronchial reaction.

CASE REPORT

The patient, B. S., 32 years old, salesman by occupation, gave a history of allergy in his family. He presented himself on July 7, 1941, with the complaint that for the past eleven years he had become incapacitated during the end of August and the first few weeks in September. During these three or four weeks he became drowsy, listless, dull, could not concentrate, his thoughts were confused; in fact, he slept most of that time. During this period his systolic blood pressure is low (60 to 80). He has no hay fever, asthma or any other usual allergic symptom. During one of these episodes he was observed at the Neurological Institute in New York with negative physical findings save the low blood pressure.

On physical examination he was well developed and nourished, appeared depressed and listless, and answered questions slowly. The blood pressure was low, 80 systolic and 50 diastolic. The blood count was negative except for 8 per cent eosinophiles. He presented himself in July because for the first time in these eleven years the symptoms began the end of June instead of late August.

On skin testing he gave a marked reaction to 1,000 units of ragweed pollen extract and a moderate reaction to 10,000 units of timothy extract. The Prausnitz-Küstner test was negative. He was given 0.4 c.c. of epinephrine hydrochloride 1:1,000 and felt much better within ten minutes.

With the timothy season at its close no treatment with timothy pollen was given, and his symptoms cleared in a week. He was treated with ragweed pollen extract

From the Department of Allergy, the Roosevelt Hospital.

twice weekly in increasing doses. During the pollen season he felt perfectly comfortable except on the days when he was overdosed. He was able to take without any ill effects 5,000 units of ragweed extract, but when 7,000 units were given his symptoms were moderately reproduced. Under this treatment, for the first time in eleven years, he was able to attend to his occupation without the loss of a single day throughout the ragweed season.

When examined after the pollen season his blood pressure was 130 systolic and 80 diastolic. Blood eosinophiles were 3 per cent.

REFERENCES

1. Rowe, A. R.: J. Lab. & Clin. Med. **8**: 13, 41, 1927.
2. Vaughan, W. T.: Virginia M. J. **56**: 735, 1930.
3. May: Quoted by Bray in Recent Advances in Allergy, ed. 2, Philadelphia, 1930, p. 384, P. Blakiston's Son & Co.
4. Rowe, A., and Richet, C.: J. Med. Francais **19**: 41, 1930.
5. Crowe, W. R.: J. ALLERGY **13**: 173, 1942.

Society Proceedings

Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 2, 1942

RECOMMENDATIONS OF THE ADVISORY BOARD OF AMERICAN ALLERGISTS ON TRAINING FOR CERTIFICATION IN ALLERGY

In view of the fact that certification in Internal Medicine and in Allergy is now an accomplished fact, the Advisory Board of American Allergists has given careful consideration to the training of prospective applicants for certification in Allergy, which is secondary to and dependent on the primary certification in Internal Medicine by the American Board of Internal Medicine.

The recommendations of the Advisory Board of American Allergists for adequate training are as follows:

1. Two years, full time, in a satisfactory allergy clinic and its hospital (including training both in allergy and internal medicine) or
2. One year, full time, in the allergy clinic and its hospital, and two additional years of full attendance on such an allergy clinic and its activities, or
3. Five years, full attendance, on such an allergy clinic and its activities, or
4. Special training under qualified preceptors, solely or in combination with aforementioned types of training, and such other training as might be felt adequate to prepare an individual for the practice of allergy.

It was the further opinion of this Board that clinics for such training should fulfill the following qualifications:

1. They should be in hospitals that have been accepted by the Council on Medical Education and Hospitals and by The American Board of Internal Medicine for training and certification in Internal Medicine and for Residencies in Medicine.
2. The Allergy Clinic should be certified by the Advisory Board of American Allergists.
3. The Director of the Allergy Clinic should be a man who is certified in Allergy by the American Board of Internal Medicine.

DISCUSSION

DR. JEROME GLASER.—It has always seemed to me that this matter of certifying as allergists only those who have the certificate of the American Board of Internal Medicine is rather hard on some of the

rest of us who have entered the field of allergy from another angle. Speaking for myself, I am a pediatrician. Two-thirds of my work is now allergy. I have the certificate of the American Board of Pediatrics and I have tried to follow the suggestion of Dr. Kern and interest the American Board of Pediatrics in the certification of pediatricians in allergy in the same way that internists are certified in allergy. They are not the least bit interested.

I believe they feel that allergy does not belong to any of the older subdivisions of medicine alone and that if allergists should be certified some special mechanism should be set up for this purpose.

As regards the importance of allergy in pediatric practice, it has been pointed out repeatedly in these meetings that the great majority of allergic symptoms begin in early youth or childhood. Since I was particularly interested in allergy at the time I started practicing pediatrics, I have had most unique opportunities in observing the natural history of allergic disease from the newborn period onward. More and more pediatricians have become interested in allergy, and as time goes on I feel that the pediatricians will be the allergists of the future and that the internists and other specialists will deal mainly with the end products of neglected opportunities in pediatric allergy.

Something should also be said for the nose and throat specialists and the dermatologists who have developed the allergic aspects of their practice to such an extent as to win general recognition as competent allergists. What of the general practitioner who has specialized in allergy and is not eligible even to take the examination of the American Board of Internal Medicine? I am sure there are many other individuals practicing allergy who are not eligible for any board examinations unless a special Board of Allergy is set up to consider all cases.

I do not feel like letting the internists so completely take over the field of allergy.

DR. VAUGHAN.—I should like to ask Dr. Cooke what would be the solution to my personal problem in view of the fact that the clinics must be in hospitals.

Our clinic is not in a hospital. We use three hospitals. Richmond has a rather unusual situation in that it is a place with several small hospitals. We have been training men for several years with a one-year course. Two of these men have put in two years.

One of these just finished. You will be interested to know his reaction to the two years' training. He said that he had learned enough more in his second year to make two years worth while.

The year of training that we have been giving has been accepted by the Board of Internal Medicine as a year toward certification, but as far as I can see the requirement that the clinic must be in a hospital would leave me out on a limb.

DR. COOKE.—First of all, the answer to Dr. Vaughan's question is covered under the fourth point on Recommendations, "Special Training Under Qualified Preceptors Solely or in Combination With Aforementioned Types of Training and Such Other Training as Might be Felt Adequate to Prepare an Individual for the Practice of Allergy." Dr. Vaughan's problem is very easily settled under that fourth recommendation.

Now, Dr. Glaser's point is one that is very well taken. The Advisory Board of the American Allergists, the former Standards Committee, had no alternative except to cooperate as they were invited to cooperate with the Board of Internal Medicine.

We realize very fully that the pediatricians and the dermatologists are under other boards and that their examinations and certifications come under those boards. It is our hope, in spite of the fact that there seems to be on the part of the pediatricians, at least, a little hesitancy at the present time. Once this thing is fully established with the Board of Internal Medicine, the Board of Pediatries will find it advantageous, especially on pressure from pediatrician allergists, to accept us as an Advisory Committee with their Board just as we now act as an Advisory Committee with the Board of Internal Medicine. The Board of Pediatrics would then grant the certification to their subspecialty allergists.

Now, these things never all happen at once and I still feel that the American Board of Pediatricians is not conversant with the situation as it exists today. They are not quite aware of the significance of the move that has been made by the internists, and they are a little bit inclined to take a stand in opposition. I believe it is only because they lack proper information, but continued pressure by the allergists in the pediatric group may bring about a changed attitude especially if we enlighten them on what we are trying to do.

Such a set-up will be to the ultimate advantage of everyone. There is no attempt to exclude the pediatricians at all, not the slightest idea of that, nor the dermatologists. The same thing holds for the laryngologists; those who are interested in allergy can qualify through their own boards in cooperation with us. All we ask is that at the time of their examination for certification some idea of their qualifications be obtained according to the recommendations just read and then proved by an oral examination. That is all that exists today as far as internal medicine is concerned.

Our only part is to recommend guest examiners who cooperate with the Board of Internal Medicine at the oral examination. They may or may not include questions on allergy in the written examination.

I would urge the pediatricians to keep insisting to their Board that they have some recognition and at least get them interested enough to be willing to try to thrash out the problem with us because we are really more than receptive to meeting them halfway or more than halfway.

Dr. Harry L. Rogers.— May I ask why it is we have to be subservient to the Board of Internal Medicine? I do not understand that. We are dealing with twelve or fifteen diseases and we have to be qualified in the study of all of these before we can come up for our certification for allergy. Are we not big enough to stand on our own legs and have our own certification independent of the Internal Medicine group?

Dr. Cooke.—I think that everyone recognizes that a fundamental training in internal medicine is necessary for adequate training in any subspecialty in medicine or pediatries. We must be fundamentally sound in medicine before we can be sound in allergy.

Medicine is, after all, the thing that we are really dealing with, and we are dealing with a particular phase of medicine. If we were going

to accept men who really had no proper fundamental training in internal medicine, we would still find the same situation that existed years ago. For instance, when I was in medical school, the medical school took high school students directly and trained them as doctors. They found eventually that it did not work. They did not have the basic scientific knowledge that was necessary for an understanding of medicine.

So it is here that I think in the early years of training a man should point to a general knowledge of the field of medicine before he attempts his especial training in any field in which he is particularly interested. That holds too with the other cooperating Boards, with the gastroenterologists, with the cardiologists, and with those whose specialty is pulmonary tuberculosis.

All these groups are acting as subspecialty groups with the Board of Internal Medicine because they realize that their early training and fundamental knowledge of medicine is an absolute prerequisite to satisfactory practice of their subspecialties.

Dr. Harold A. Abramson.—There is one group to which I belong which, perhaps, has not been mentioned. That is the group who after a medical internship in an approved institution, has spent many years, in my case ten, in full-time academic medicine, specializing for the most part in immunology and biochemistry. I do not think that any of us could doubt that immunology and biochemistry are important for progress in allergy. In spite of a fairly good training in the scientific disciplines of medicine, it would be very hard for me at present to qualify for the very many questions which might be asked by the Board of Internal Medicine. In these times when the energies of all of us should and must be directed toward helping solve problems connected with the military situation, it is difficult to prepare for questions which we have answered on examinations and papers many times indeed. I confess I would have a hard time answering some of those that I have seen. Do you not think that under present conditions if physicians whose qualifications make them acceptable for examination and whose training has been under proper auspices in internal medicine, pediatrics, immunology, or other specialty should be certified without examination as allergists if they have had, in addition, the proper training in allergy?

While revising this discussion, I note the following paragraph in the editorial column of the May 16, 1942 issue of the Journal of the American Association.

> Today the situation is much more complicated than in the times of previous wars. A system of extended residencies and assistantships associated with qualification for the certificates of the boards in the specialties now covers many young graduates in medicine. Especially needed at this time as a means of encouragement to such young men in offering their services to the armed forces is a definite action on the part of each one of the certifying boards indicating the extent to which it will accept military service as a part of the requirement for certification in a specialty of medical practice.

Dr. Cooke.—Dr. Abramson is an exception to the rule. I think there are very few here, we will say, who have not practiced medicine, who went from an internship into academic life for any number of years.

It presents a problem. I really do not know how to answer him except that after all is said and done it is true that now that he has come back into the clinic field, of course, he can acquire a knowledge of clinical medicine which did not interest him particularly for a period of ten years. There are not very many men who have the mental capacity to do that, but I am sure he has. Yet, I do not quite know what the answer is. I think it is one that the Board should give consideration to and it is possible that there will be a solution worked out.

At the same time, I think his particular personal problem is such an exception to the rule that it must be regarded as one that makes the rule desirable. I do not quite know what the answer is going to be but I think it should and will receive the attention of the Board.

The recommendations of the Advisory Board were approved by the Society.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND
THEODORE T. BERNSTEIN, M.D., CHICAGO

Further Observations on the Treatment of Intractable Bronchial Asthma by Bilateral Resection of the Pulmonary Plexus. Gay, L. N., and Rienhoff, W. M.: Bull. Johns Hopkins Hosp. **70**: 386, 1942.

Bilateral resection of the pulmonary plexus was done in twenty-one patients with intractable asthma. Eight derived marked benefit from the operation, five died either as a result of the operation or shortly afterward, four died later, and four were unimproved. Neither the age of the patient nor the duration of the asthma had any relation to the outcome of the operation.

Oral Treatment of Hay Fever With Ragweed Pollen, Iliff, E. H., and Gay, L. N.: Bull. Johns Hopkins Hosp. **70**: 378, 1942.

An analysis of the results of oral pollen therapy during 1940 is reported. Of eighty-two cases treated by this method, sixty-two were treated preseasonally and twenty coseasonally. Of the orally treated cases, excellent results were obtained in 4.8 per cent of the preseasonal group and in none of the coseasonal group. The hypodermically treated cases, on the other hand, showed excellent results in 13 per cent. Good results were noted in 16.1 per cent of the preseasonal oral cases, none in the coseasonal oral, and 29.7 per cent in the hypodermically treated group. Fair results are reported in a large percentage of those orally treated. The interpretation of what is ''fair'' is, however, open to some question, since in the coseasonal oral group 70 per cent had ''fair'' results while none had excellent or good effects. Reactions were present in a large percentage, most of them gastrointestinal. Reagin studies failed to show any decisive changes. The authors conclude that while hypodermic treatment is the most effective method, oral treatment is justifiable in special circumstances.

Effects of Cocaine and Sympathetomimetic Amines on Humoral Transmission of Sympathetic Nerve Actions. Lawrence, W. S., Morton, M. C., and Tainter, M. L.: J. Pharmacol. & Exper. Therap. **75**: 219, 1942.

The authors find that in a perfusion of the vascular bed of the cat's isolated hind leg the addition of cocaine to epinephrine diminishes the rate of destruction of the latter. When the sympathetic nerves to the leg are allowed to degenerate and the perfusion is carried on with diluted defibrinated oxblood, the pressor effect of epinephrine, cobefrine, and neosynephrine is increased several times. This enhancement of pressor effect is not produced with ephedrine or tyramine under the same conditions. According to the authors, these results lend further support to the prevailing theories of humoral transmission of sympathetic nerve impulses to blood vessels. It is conceived that ephedrine, tyramine, and cocaine block the

ferments within the smooth muscle cell, which inactivates the epinephrine entering the cell from the blood stream, or the sympathin derived intermittently from the local sympathetic nerve endings. Previous cocainization may prevent the pressor action of ephedrine or tyramine by blocking the ferment through which they normally act. The permeability of the muscle cells is increased by the degeneration of the sympathetic nerves, so that more epinephrine enters the cells, causing an increased pressor effect. This same increased permeability does not increase the pressor effects of tyramine or ephedrine.

The Effect of Exercise and of Four Commonly Used Drugs on the Normal Human Electrocardiogram, With Particular Reference to T Wave Changes. Hartwell, A. S., Burrett, J. B., Graybiel, A., and White, P. D.: J. Clin. Investigation **21**: 409, 1942.

Epinephrine lowers the T wave in the electrocardiogram of normal persons. This effect lasts from fifteen to thirty minutes. Atropine also lowers the T wave, which effect is maximal in sixty minutes but may last for ninety minutes. Mecholyl also causes a lowering of the T wave. Exercise lowers the T wave with a return to normal in less than a minute. The T wave was elevated by ergotamine tartrate or pressure on the right carotid sinus. The authors call attention to the significance of these findings when electrocardiograms are interpreted.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND
MURRAY ALBERT, M.D., BROOKLYN

Species-Specific Precipitins in Serum Sickness After Intravenous Injection of Refined, Concentrated Antipneumococcal Rabbit Serum. De Gara, P. F., and Bullowa, J. G. M.: J. Immunol. **44**: 259, 1942.

The sera of twenty pneumonia patients who developed serum sickness as a result of intravenous injections of refined, concentrated antipneumococcal rabbit serum were titrated for the presence of species-specific precipitins for rabbit protein. The object of these studies was to determine whether the presence of precipitins is diagnostic of serum sickness. The sera were examined for the presence of precipitins at various intervals after serum treatment. Species-specific precipitins for rabbit protein were detected in the sera of ten of the twenty patients who developed serum sickness. In one patient precipitins were found on the sixth day following serum treatment, but in the majority of cases they appeared between the twelfth and eighteenth day after the initial serum injection, at a time when the symptoms of serum sickness had disappeared. Precipitins were not found in the sera of twenty control patients who also received antipneumococcal serum for pneumonia but who failed to show symptoms of serum sickness. There was no apparent relationship between the amount of serum injected and the presence of precipitins. These findings tend to confirm those of Tuft and Ramsdell, who demonstrated the presence of precipitins in cases of serum sickness following the injection of horse serum. Hence, the presence of precipitins may confirm the diagnosis of serum sickness but the absence of precipitins does not exclude it.

Antibodies and The Protein Reserves. Cannon, P. R.: J. Immunol. **44**: 107, 1942.

Antibodies are assumed to be molecules of globulin which, in the course of their synthesis in the cells, have been specifically modified by antigen. Antigens are

taken up from the tissue fluids by macrophages and other types of cells. Antibodies are synthesized in these cells and are then excreted into the blood or lymph or remain in the cells as tissue antibodies. Some molecules of antigen may remain within the antibody-producing cells and may serve as patterns for the synthesis of fresh antibodies.

The author believes that if antibodies are specifically modified globulins, then antibody production is directly related to normal globulin production. Globulin is synthesized from amino acids which are furnished by food proteins. A high protein diet favors the formation of globulin, whereas a diet deficient in protein reduces the globulin reserve in the cells. When the latter is diminished, antibody-globulin is withdrawn from the antibody-producing cells and is used up by the metabolic processes of the body. The need for an adequate amount of protein in the diet in order that antibody-globulin be synthesized is apparent.

During the last war it was established that the so-called war edema in the starving European population was due to hypoproteinemia, resulting from insufficient protein in the diet. Persons suffering from this condition were found to be extremely susceptible to all infections. Experiments in progress in the author's laboratory indicate that a reduction in protein reserves and a low protein diet inhibit the antibody-producing capacity of rabbits. It is concluded that the production of antibodies is directly dependent upon the protein reserves of the body.

The Role of Hypersensitivity in Periarteritis Nodosa. Rich, A. R.: Bull. Johns Hopkins Hosp. **71**: 123, 1942.

Vascular lesions typical of periarteritis nodosa were observed in four successive autopsies performed on patients who had had serum sickness shortly before death. Three patients died from lobar pneumonia and the fourth from influenzal meningitis. Each patient had received sulfonamide therapy in addition to antiserum. At autopsy, fresh lesions of periarteritis nodosa were found, despite the fact that the patients had not exhibited any characteristic clinical symptoms of this condition. A biopsy was performed on a fifth patient who developed serum sickness following the administration of antipneumococcus serum and manifested symptoms suggestive of periarteritis nodosa. Periarterial inflammatory infiltration with polymorphonuclear and mononuclear cells was observed in a section of biceps muscle tissue. This patient had received sulfonamide therapy in addition to serum. Evidence of periarterial infiltration was observed in two additional autopsies; one patient had received only sulfathiazole, the other had received only antiserum. The vascular lesions found in the seven autopsies described consisted of necrosis, fibrinoid alteration, hyalinization of the media of the involved arteries, perivascular infiltration, or infiltration of the entire wall with mononuclear and polymorphonuclear leucocytes. Eosinophilic infiltration was present in the majority of the cases.

It was concluded from these findings that a definite relationship exists between the hypersensitive reactions and the vascular lesions, since the lesions were fresh and none of the patients had manifested clinical symptoms of periarteritis nodosa before the onset of their fatal infections. Evidence is cited to indicate that the vascular lesions, in all probability, were related to the hypersensitive reaction which is responsible for serum sickness rather than to the fatal infection or to sulfonamide therapy. However, sulfonamides alone can cause vascular lesions of periarteritis. A review of the literature suggests that sulfonamides cause these lesions as a result of a hypersensitiveness rather than as a result of intoxication. The sulfonamides can attach themselves to plasma proteins and act as haptens. The author believes that the findings in the seven autopsies described and the experimental evidence reported in the literature indicate that periarteritis nodosa

is a manifestation of hypersensitiveness. The vascular lesions may be caused by a number of antigens. However, the possibility that toxic agents can cause perivascular lesions by direct action has not been completely excluded. It is suggested that in cases in which a clinical diagnosis of periarteritis nodosa is tenable, a thorough investigation should be made for the offending antigen. Its elimination may lead to a regression of the lesions.

Influence of an Ethylendiamine Derivative on Histamine Intoxication and Anaphylactic Shock in the Intact Guinea Pig and the Isolated Guinea Pig Heart. Wilcox, H. B., Jr., and Seegal, E. C.: J. Immunol. **44**: 219, 1942.

The inhibitory effect of N-phenyl-N-ethyl-N'-diethylethylendiamine (1571F) on histamine intoxication and anaphylactic shock in guinea pigs was investigated. A series of fifteen guinea pigs received intravenous injections of 1571F ten to twenty minutes before the intravenous injection of histamine. Eleven animals were sufficiently protected to survive one to six minimal lethal doses of histamine. Most of the animals, however, exhibited severe shock. The degree of protection varied with the amount of 1571F injected. In twenty-one guinea pigs sensitized to horse serum, 1571F given intravenously ten to twenty minutes prior to the intravenous injection of one to four shocking doses of antigen prevented death in all but four animals. However, almost all animals exhibited symptoms of shock.

The effect of 1571F on the histamine and anaphylactic reactions of the isolated, perfused guinea pig heart was also studied. Histamine, when perfused through the guinea pig heart, causes a transitory reduction in the rate of perfusion through the coronary vessels and a temporary increase in rate and amplitude of contraction. When 1571F was present in the perfusion fluid, the addition of histamine to the perfusate was followed by an increase in perfusion rate instead of a decrease. The other histamine effects were unchanged. In specifically sensitized hearts, perfusion with a solution containing the antigen produces a transient reaction consisting of an increase in the rate and amplitude of contraction and a marked decrease in the rate of flow of the perfusate through the coronary vessels. In the presence of 1571F, the addition of the specific antigen failed to cause the usual decrease in rate of coronary flow. The increase in the rate and amplitude of contraction were not appreciably affected by 1571F. Hence, 1571F inhibits fatal reactions but does not prevent the development of symptoms in histamine and anaphylactic shock. The addition of 1571F to the perfusion fluid of the isolated heart prevents the usual decrease in rate of coronary flow in histamine or anaphylactic shock but does not inhibit the increase in rate and amplitude of contraction of the heart. Apparently 1571F acts by preventing the contraction of smooth muscle.

Evidence for the Existence of Two Antibodies for Crystalline Insulin. Lowell, F. C.: Proc. Soc. Exper. Biol. & Med. **50**: 167, 1942.

Studies were made on a patient who was both allergic and resistant to crystalline insulin. The intravenous injection of 30 units of a solution of zinc-insulin crystals derived from beef and pork pancreas failed to alter appreciably the patient's fasting blood sugar which varied between 330 and 380 mg. per 100 c.c. However, an intravenous injection of 30 units of human insulin produced a marked hypoglycemia about ninety minutes afterward. Allergic reactions, consisting of generalized urticaria and a feeling of constriction in the throat, occurred within a few minutes following the injection of either human or crystalline insulin. Passive transfer tests were performed by injecting the patient's serum alone or mixtures of this serum with crystalline insulin into the skin of normal recipients. The sites were subsequently tested with either crystalline or human

insulin. Skin-sensitizing antibodies for human and animal insulins could be demonstrated in ten specimens of serum obtained from the patient over a period of several months. The skin-sensitizing activity of the serum was inhibited when adequate amounts of crystalline insulin were mixed with serum prior to sensitization. The skin-sensitizing activity of serum was destroyed when the serum was heated. Tests for insulin-neutralizing antibody were made by mouse assay. A dose of 0.02 unit of insulin caused hypoglycemic shock in 90 to 100 per cent of the mice. The presence of an insulin-neutralizing antibody was demonstrated when the patient's serum was mixed with crystalline insulin. However, no insulin-neutralizing effect was demonstrable when human instead of crystalline insulin was employed. Heating of the patient's serum failed to destroy its insulin-neutralizing activity. From these studies it was concluded that two antibodies were present in the patient's sera: a skin-sensitizing heat-labile antibody and a heat-stable insulin-neutralizing antibody. The latter probably accounted for the patient's resistance to insulin. The fact that the patient was not resistant to human insulin indicates that the neutralizing antibody is not an antihormone. The patient's resistance to insulin was apparently unrelated to the allergic phenomena since both crystalline and human insulin caused allergic reactions.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, SAN FRANCISCO

The Relation of Vasomotor Rhinitis to Bronchial Asthma. Urbach, E., and Gottlieb, P. M.: Arch. Pediat. **59**: 382, 1942.

This report is based on 379 cases of asthma seen at the University of Vienna. The age distribution is not stated. Only 12 per cent were considered to be due to specific allergens, 10 per cent were apparently associated with infection, while the rest were considered to be pathergic rather than allergic; i.e., due to such unspecific causes as dust, wind, cold, and excitement.

Thirty-eight per cent of cases had vasomotor rhinitis as well as asthma. Where both conditions appeared simultaneously, there was a common etiology. This was also apt to be the case where they developed within two years of each other. If more than two years separated the appearance of asthma from the appearance of vasomotor rhinitis, different etiologies were to be expected.

Gastrointestinal Allergy and the Celiac Syndrome. Kunstader, R. H.: J. Pediat. **21**: 193, 1942.

Three instances of what appeared to be the celiac syndrome in which cow's milk was the chief cause of the chronic diarrhea are presented. Two were children of 18 months of age, and the third a child of 19 months of age at the time milk was eliminated. There was immediate improvement in the foul-smelling large stools containing increased fat, and in the matter of abdominal distension, loss of weight, irritability, and anorexia. In two of the cases there was a history of allergy in the family as well as eczema in the patient. In all three of the children other food allergies or intolerances were present, and in two, intradermal tests of milk were done and were negative. None of the patients had detailed laboratory studies designed to confirm the diagnosis of celiac disease. It is suggested that before these are done in the management of celiac disease, milk elimination should be tried, regardless of the outcome of the skin tests.

Serum Sickness and Anaphylaxis: Analysis of Cases of 6,211 Patients Treated With Horse Serum for Various Infections. Kojis F. G.: Am. J. Dis. Child. **64**: 93 and 313, 1942.

Most of the patients dealt with in this study were seen by the author at Willard Parker Hospital in New York City between 1928 and 1932. The mortality from serum anaphylaxis in the series was 1 in 1,242. This is much greater than Park's frequently quoted figure of 1 in 50,000 which appears to have been derived from a study of the literature.

Various factors influence the incidence of serum sickness and anaphylaxis, including the kind of serum, its method of preparation, the quantity, the horse producing it, the race of the patient (Negroes less susceptible than whites), the route of administration, and the patient. The most important factors are the kind of serum, the method of preparing it, the repetition of injections and the patient.

When the reaction to the intradermal sensitivity test was positive, the incidence of serum sickness following inoculation of diphtheria antitoxin was four times, of anaphylaxis, thirty-five times, and of death, eleven times greater than when the reaction to the test was negative. If the reaction to the conjunctival test was positive, the incidence of serum sickness was five times and of anaphylaxis, one hundred seventy-three times greater.

Secondary injections increased the incidence of serum sickness by 50 per cent while the anaphylaxis rate was twenty-three times greater than after the primary injections. This figure of twenty-three is derived from fourteenfold increase where the intramuscular route was used and a sixty-twofold increase when the intravenous route was used.

Seven types of serum rash are described, including twenty-four different signs and symptoms. The five deaths reported each followed a secondary injection. In the two instances in which an autopsy was performed, the findings were typical of anaphylaxis.

Thermal reactions were found to be dependent on the horse producing the serum.

Round Table Discussion on Allergy. J. Pediat. **21**: 113, 1942.

This discussion by Doctors Walzer, Rackemann, Donnally, Pratt, Ratner, and Tuft brings out both general principals in which the discussants are agreed as well as points upon which each feels more emphasis should be placed. While perennial treatment of pollinosis appears to be accepted as the most satisfactory type of treatment, a difference of opinion develops in the matter of treating an individual with positive tests but a negative clinical history. The part played by foods, especially corn, cherries, chocolate, and alcohol, in aggravating pollinosis is discussed. It is suggested that determination of the level of an individual's constitutional reaction may provide a short cut in treatment. The concept of an allergenically denatured diet is presented. The value of ipecac used as an emetic in obstructive asthma is brought out. In addition, serum allergy, history taking, and numerous other subjects are discussed.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Sulfathiazole in the Treatment of Sinusitis. Marks, R. F.: Arch. Otolaryng. **35**: 794, 1942.

Marks presents a review of the literature and a report of ten cases of chronic maxillary sinusitis in which sulfanilamide and sulfathiazole were employed. He

found that sulfathiazole jelly, 20 or 50 per cent, was an effective therapeutic agent when injected into the maxillary sinus in which there was evidence of chronic maxillary sinusitis. A suspension of sulfathiazole seemed to be more effective than sodium sulfathiazole when used clinically. The average duration of symptoms among the ten patients was ten months, and the average time required for resolution was about three or four weeks. This suggests that a trial with sulfathiazole is indicated in the treatment of chronic maxillary sinusitis before surgical treatment is advised.

Experimental Hypersensitivity of Mucous Membranes of the Upper Respiratory Tract. Frank, I., Blahd, M., and Howell, K. M.: Arch. Otolaryng. 35: 918, 1942.

Frank, Blahd, and Howell report their observations on experimental hypersensitivity of mucous membranes of the upper respiratory tract. They found that the mucous membranes can be sensitized via many portals, including other mucous membranes. They noted that foreign substances introduced into the nasal sinus, without injury to the mucous membrane, sensitized distant areas, such as the skin and other mucous membranes.

Vasomotor Rhinitis in the Tropics. Jaffe, Ludwig: Arch. Otolaryng. 35: 47, 1942.

Jaffe reports his observations on vasomotor rhinitis in the tropics. He found that the condition is quite common in this climate. There was a great deal of dust, especially in the cities, and it was felt that this was a factor in the cause of the trouble. No atmospheric studies were made in regard to pollens or mold spores. No skin tests or allergic management were instituted. The patients were treated by cauterization of the mucous membrane with a concentrated solution of trichloracetic acid. The author warns against unnecessary operations. He suggests methods of conservative therapy which he has found to be satisfactory.

Prevention of Death in Status Asthmaticus: Value of Bronchoscopy. Bases, L., and Kurtin, A.: Arch. Otolaryng. 35: 79, 1942.

Bases and Kurtin emphasize the value of bronchoscopy in the prevention of death in *status asthmaticus*. They report that six of the seven patients who died of *status asthmaticus* at the Mount Sinai Hospital and on whom autopsy was performed in the past fifteen years died from blocking of the tracheobronchial airway by excessive outpouring of secretion. They reviewed the literature on death in asthma and pointed out the frequency with which it was noted that obstruction of the tracheobronchial tree with secretion was present. They give a case report of an instance in which bronchoscopic aspiration was a life-saving procedure. They conclude in saying that the most important factor in causing death in *status asthmaticus* is obstruction of the tracheobronchial tree by viscid secretion. They point out that this material can be removed by suction through a bronchoscope. They strongly advise the use of this procedure in those cases of *status asthmaticus* in which other measures have proved unsuccessful.

Presence and Action of Lysozyme in the Nasal Mucus. Bronner, C. E. C.: Tr. Chicago Laryng. & Otol. Soc., Oct. 6, 1941. (Arch. Otolaryng. 35: 825, 1942.)

Bronner reports his observations on the presence and action of lysozyme in the nasal mucosa. He states that in addition to the mechanical action of the ciliated epithelium and the drainage, lysozyme can be considered a factor in the mechanism of local tissue resistance. He found that lysozyme disappeared or decreased

notably during the first two to four days of a common cold. At that time *M. lysodeikticus*, sarcinas, or other types of spore-bearing rods which were easily dissolved by secretion from healthy noses were cultivated from the nasal mucosa or from the mucus. These organisms evidently could not be found in the nose if lysozyme was present. He further states that this lack of lysozyme in a running nose holds true only for the common cold. The discharge from the running nose of hay fever patients shows a high lysozyme content. Reasons that the absence could not be considered as proof of a causal connection between the common cold and lysozyme were discussed.

Correspondence

The Editor,
THE JOURNAL OF ALLERGY.

At the recent meeting of the Medical Society of the District of Columbia (September 29 to October 1) a completely equipped emergency medical field set was donated to Vice Admiral R. R. Waesche of the U. S. Coast Guard by the Medical and Surgical Relief Committee of America, a relief organization with headquarters at 420 Lexington Ave., New York, N. Y. Ten similar sets have been turned over to the Coast Guard, bringing the total number of sets furnished by the Committee to first-aid posts, emergency medical centers, needy hospitals, and other recognized relief agencies up to 233.

Each of the sets consists of two portable cases completely equipped with instruments, medicines, antiseptics, and a lantern with a set of dry cells to provide power for lights in the event of blackouts or power difficulties. The sets were designed by physicians after a series of experiments during the bombing attacks on London.

The campaign to furnish emergency medical field sets is only one phase of the work in which the Committee has been actively engaged since its inception in August, 1940. Organized by Mrs. Millicent Huttleston Rogers and a few New York City physicians to send surplus medicines and surgical instruments to physicians in Great Britain, it has since grown to a nationwide committee consisting of approximately 500 physicians and surgeons assisted by an equal number of professional and nonprofessional volunteers.

As the war spread, the organization's scope was broadened to take care of requests from Greece, China, Russia, the Royal Norwegian Forces in Iceland, and the Free French Forces in Northern Africa and Miquelon. Since Pearl Harbor, primary consideration has been given to America's needs.

To date, medical supplies and surgical instruments valued at $475,690.05 have been sent out by the Committee. The supplies are collected from physicians, hospitals, pharmaceutical houses, and drugstores, then sent to the Committee's New York headquarters. Medicines are sorted and rebottled under the supervision of trained nurses. Instruments are polished, and, if necessary, reconditioned. They are then redistributed to organizations where the need for them exists.

MEDICAL AND SURGICAL RELIEF COMMITTEE OF AMERICA.

420 Lexington Avenue
New York City
Oct. 1, 1942